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(57) Abstract

The invention provides BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptides, and polynucleotides encoding BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptides, and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses.

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Novel Compounds

FIELD OF THE INVENTION

This invention relates to polynucleotides, (herein referred to as "BASB051 polynucleotide(s)", "BASB057 polynucleotide(s)", "BASB060 polynucleotide(s)", "BASB061 polynucleotide(s)", "BASB063 polynucleotide(s)", "BASB065 polynucleotide(s)", "BASB066 polynucleotide(s)" and "BASB071 polynucleotide(s)"), polypeptides encoded by them (referred to herein as "BASB051", "BASB057", "BASB060", "BASB061", "BASB063", "BASB065", "BASB066" and "BASB071" respectively or "BASB051 polypeptide(s)", BASB057 polypeptide(s)", BASB060 polypeptide(s)", BASB061 polypeptide(s)", BASB063 polypeptide(s)", BASB065 polypeptide(s)", BASB066 polypeptide(s)" and BASB071 polypeptide(s)" respectively), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including vaccines against bacterial infections. In a further aspect, the invention relates to diagnostic assays for detecting infection of certain pathogens.

BACKGROUND OF THE INVENTION

Neisseria meningitidis (meningococcus) is a Gram-negative bacterium frequently isolated from the human upper respiratory tract. It occasionally causes invasive bacterial diseases such as bacteremia and meningitis. The incidence of meningococcal disease shows geographical seasonal and annual differences (Schwartz, B., Moore, P.S., Broome, C.V.; Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Most disease in temperate countries is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total population sometimes reaching higher values (Kaczmarski, E.B. (1997), Commun. Dis. Rep. Rev. 7: R55-9, 1995; Scholten, R.J.P.M., Bijlmer, H.A., Poolman, J.T. et al. Clin. Infect. Dis. 16: 237-246, 1993; Cruz, C., Pavez, G., Aguilar, E., et al. Epidemiol. Infect. 105: 119-126, 1990).

Epidemics dominated by serogroup A meningococci, mostly in central Africa, are encountered, sometimes reaching levels up to 1000/100.000/year (Schwartz, B., Moore, P.S., Broome, C.V. Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Nearly all cases as a whole of meningococcal disease are caused by serogroup A, B, C, W-135 and Y meningococci and a tetravalent A, C, W-135, Y polysaccharide vaccine is available (Armand, J., Arminjon, F., Mynard, M.C., Lafaix, C., J. Biol. Stand. 10: 335-339, 1982).

The polysaccharide vaccines are currently being improved by way of chemical conjugating them to carrier proteins (Lieberman, J.M., Chiu, S.S., Wong, V.K., et al. JAMA 275 : 1499-1503, 1996).

A serogroup B vaccine is not available, since the B capsular polysaccharide was found to be nonimmunogenic, most likely because it shares structural similarity to host components (Wyle, F.A., Artenstein, M.S., Brandt, M.L. et al. J. Infect. Dis. 126: 514-522, 1972; Finne, J.M., Leinonen, M., Mäkelä, P.M. Lancet ii.: 355-357, 1983).

For many years efforts have been initiated and carried out to develop meningococcal outer membrane based vaccines (de Moraes, J.C., Perkins, B., Camargo, M.C. et al. Lancet 340: 1074-1078, 1992; Bjune, G., Hoiby, E.A. Gronnesby, J.K. et al. 338: 1093-1096, 1991). Such vaccines have demonstrated efficacies from 57% - 85% in older children (>4 years) and adolescents.

Many bacterial outer membrane components are present in these vaccines, such as PorA, PorB, Rmp, Opc, Opa, FrpB and the contribution of these components to the observed protection still needs futher definition. Other bacterial outer membrane components have been defined by using animal or human antibodies to be potentially relevant to the induction of protective immunity, such as TbpB and NspA (Martin, D., Cadieux, N., Hamel, J., Brodeux, B.R., J. Exp. Med. 185: 1173-1183, 1997; Lissolo, L., Maître-Wilmotte, C., Dumas, p. et al.,

Inf. Immun. 63: 884-890, 1995). The mechanisms of protective immunity will involve antibody mediated bactericidal activity and opsonophagocytosis.

A bacteremia animal model has been used to combine all antibody mediated mechanisms (Saukkonen, K., Leinonen, M., Abdillahi, H. Poolman, J. T. Vaccine 7: 325-328, 1989). It is generally accepted that the late complement component mediated bactericidal mechanism is crucial for immunity against meningococcal disease (Ross, S.C., Rosenthal P.J., Berberic, H.M., Densen, P. J. Infect. Dis. 155: 1266-1275, 1987).

The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Neisseria meningitidis* strains that are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

SUMMARY OF THE INVENTION

The present invention relates to BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071, in particular BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 polypeptides and BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial diseases, amongst others. In a further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 polynucleotides or polypeptides.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The invention relates to BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 polypeptides and polynucleotides as described in greater detail below. The invention relates especially to BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066, and BASB071 having the nucleotide and amino acid sequences set out in SEQ ID NO:1,3,5,7,9,11,13,15 and SEQ ID NO:2,4,6,8,10,12,14,16 respectively. It is understood that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of one embodiment of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

Polypeptides

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis* referred to herein as "BASB051", "BASB057", "BASB060", "BASB061", "BASB063", "BASB065", "BASB066" and "BASB071", and "BASB051 polypeptides", "BASB057 polypeptides", "BASB060 polypeptides", "BASB061 polypeptides", "BASB063 polypeptides", "BASB065 polypeptides", "BASB066 polypeptides", and "BASB071 polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2.

The BASB051 polypeptide provided in SEQ ID NO:2 is the BASB051 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB051 polypeptide, that is, a contiguous portion of the BASB051 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB051 polypeptide. Such an immunogenic fragment may include, for example, the BASB051 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB051 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:4.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:4

The BASB057 polypeptide provided in SEQ ID NO:4 is the BASB057 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB057 polypeptide, that is, a contiguous portion of the BASB057 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:4. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB057 polypeptide. Such an immunogenic fragment may include, for example, the BASB057 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB057 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:4 over the entire length of SEQ ID NO:4.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:6.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:5 over the entire length of SEQ ID NO:5.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:6

The BASB060 polypeptide provided in SEQ ID NO:6 is the BASB060 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB060 polypeptide, that is, a contiguous portion of the BASB060 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:6. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB060 polypeptide. Such an immunogenic fragment may include, for example, the BASB060 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB060 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:6 over the entire length of SEQ ID NO:6.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:8.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:7 over the entire length of SEQ ID NO:7.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:8

The BASB061 polypeptide provided in SEQ ID NO:8 is the BASB061 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB061 polypeptide, that is, a contiguous portion of the BASB061 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:8. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB061 polypeptide. Such an immunogenic fragment may include, for example, the BASB061 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB061 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:8 over the entire length of SEQ ID NO:8.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:10.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:9 over the entire length of SEQ ID NO:9.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:10

The BASB063 polypeptide provided in SEQ ID NO:10 is the BASB063 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB063 polypeptide, that is, a contiguous portion of the BASB063 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:10. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB063 polypeptide. Such an immunogenic fragment may include, for example, the BASB063 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB063 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:10 over the entire length of SEQ ID NO:10.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:12.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:11 over the entire length of SEQ ID NO:11.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:12

The BASB065 polypeptide provided in SEQ ID NO:12 is the BASB065 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB065 polypeptide, that is, a contiguous portion of the BASB065 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:12. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB065 polypeptide. Such an immunogenic fragment may include, for example, the BASB065 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB065 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:12 over the entire length of SEQ ID NO:12.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:14.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:13 over the entire length of SEQ ID NO:13.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:14

The BASB066 polypeptide provided in SEQ ID NO:14 is the BASB066 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB066 polypeptide, that is, a contiguous portion of the BASB066 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:14. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB066 polypeptide. Such an immunogenic fragment may include, for example, the BASB066 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB066 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:14 over the entire length of SEQ ID NO:14.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:16.

(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:15 over the entire length of SEQ ID NO:15.

(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:16

The BASB071 polypeptide provided in SEQ ID NO:16 is the BASB071 polypeptide from *Neisseria meningitidis* strain ATCC13090.

The invention also provides an immunogenic fragment of a BASB071 polypeptide, that is, a contiguous portion of the BASB071 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:16. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB071 polypeptide. Such an immunogenic fragment may include, for example, the BASB071 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB071 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:16 over the entire length of SEQ ID NO:16.

A fragment is a polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention. As with

BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of SEQ ID NO:2,4,6,8,10,12,14,16 or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO:2,4,6,8,10,12,14,16 or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO:2,4,6,8,10,12,14,16.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these fragments may be employed as intermediates for producing the full-length polypeptides of the invention.

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenzae virus, NS1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E.coli* C-LytA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

It is most preferred that a polypeptide of the invention is derived from *Neisseria meningitidis*, however, it may preferably be obtained from other organisms of the same taxonomic genus.

A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

Polynucleotides

It is an object of the invention to provide polynucleotides that encode BASB051 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB051.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB051 polypeptides comprising a sequence set out in SEQ ID NO:1 which includes a full length gene, or a variant thereof.

The BASB051 polynucleotide provided in SEQ ID NO:1 is the BASB051 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB051 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB051 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB051 polypeptide having a deduced amino acid sequence of SEQ ID NO:2 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB051 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:2 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1 a polynucleotide of the invention encoding BASB051 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:1 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:1 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 802 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO: 1 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO: 1. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as

rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB051 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 801 of SEQ ID NO:1. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB051 having an amino acid sequence set out in SEQ ID NO:2. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB051 variants, that have the amino acid sequence of BASB051 polypeptide of SEQ ID NO:2 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB051 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB051 polypeptide having an amino acid sequence set out in SEQ ID NO:2 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:1.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB051 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:1.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean

hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB051 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB051 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB051 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB057 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB057.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB057 polypeptides comprising a sequence set out in SEQ ID NO:3 which includes a full length gene, or a variant thereof.

The BASB057 polynucleotide provided in SEQ ID NO:3 is the BASB057 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB057 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB057 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB057 polypeptide having a deduced amino acid sequence of SEQ ID NO:4 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB057 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:4 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:3 a polynucleotide of the invention encoding BASB057 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:3 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:3 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:3 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:4 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1402 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:4 over the entire length of SEQ ID NO:4.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:3 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:3. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB057 polypeptide of SEQ ID NO:4 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 1401 of SEQ ID NO:3. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:4. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB057 having an amino acid sequence set out in SEQ ID NO:4. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:4. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB057 variants, that have the amino acid sequence of BASB057 polypeptide of SEQ ID NO:4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB057 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB057 polypeptide having an amino acid sequence set out in SEQ ID NO:4 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:3.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB057 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:3.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:3 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB057 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB057 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB057 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB060 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB060.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB060 polypeptides comprising a sequence set out in SEQ ID NO:5 which includes a full length gene, or a variant thereof.

The BASB060 polynucleotide provided in SEQ ID NO:5 is the BASB060 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB060 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB060 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB060 polypeptide having a deduced amino acid sequence of SEQ ID NO:6 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB060 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:6 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:5 a polynucleotide of the invention encoding BASB060 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:5 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:5 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:5 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:6 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:5, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 418 of SEQ ID NO:5, encodes the polypeptide of SEQ ID NO:6.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:5 over the entire length of SEQ ID NO:5; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:6 over the entire length of SEQ ID NO:6.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:5 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:5. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB060 polypeptide of SEQ ID NO:6 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 417 of SEQ ID NO:5. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:6. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB060 having an amino acid sequence set out in SEQ ID NO:6. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:6. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB060 variants, that have the amino acid sequence of BASB060 polypeptide of SEQ ID NO:6 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions that do not alter the properties and activities of BASB060 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB060 polypeptide having an amino acid sequence set out in SEQ ID NO:6 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:5.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB060 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:5.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:5 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:5 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB060 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB060 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB060 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:5 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB061 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB061.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB061 polypeptides comprising a sequence set out in SEQ ID NO:7 which includes a full length gene, or a variant thereof.

The BASB061 polynucleotide provided in SEQ ID NO:7 is the BASB061 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB061 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB061 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB061 polypeptide having a deduced amino acid sequence of SEQ ID NO:8 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB061 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:8 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:7 a polynucleotide of the invention encoding BASB061 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:7 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:7 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:7 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:8 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:7, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 514 of SEQ ID NO:7, encodes the polypeptide of SEQ ID NO:8.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:7 over the entire length of SEQ ID NO:7; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:8 over the entire length of SEQ ID NO:8.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:7 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:7. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB061 polypeptide of SEQ ID NO:8 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 513 of SEQ ID NO:7. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:8. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB061 having an amino acid sequence set out in SEQ ID NO:8. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:8. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB061 variants, that have the amino acid sequence of BASB061 polypeptide of SEQ ID NO:8 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions that do not alter the properties and activities of BASB061 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB061 polypeptide having an amino acid sequence set out in SEQ ID NO:8 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:7.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB061 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:7.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:7 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:7 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB061 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB061 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB061 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:7 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB063 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB063.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB063 polypeptides comprising a sequence set out in SEQ ID NO:9 which includes a full length gene, or a variant thereof.

The BASB063 polynucleotide provided in SEQ ID NO:9 is the BASB063 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB063 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB063 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB063 polypeptide having a deduced amino acid sequence of SEQ ID NO:10 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB063 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:10 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:9 a polynucleotide of the invention encoding BASB063 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:9 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:9 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:9 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:10 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:9, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 814 of SEQ ID NO:9, encodes the polypeptide of SEQ ID NO:10.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:9 over the entire length of SEQ ID NO:9; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:10 over the entire length of SEQ ID NO:10.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:9 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:9. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB063 polypeptide of SEQ ID NO:10 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 813 of SEQ ID NO:9. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:10. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB063 having an amino acid sequence set out in SEQ ID NO:10. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:10. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB063 variants, that have the amino acid sequence of BASB063 polypeptide of SEQ ID NO:10 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions that do not alter the properties and activities of BASB063 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB063 polypeptide having an amino acid sequence set out in SEQ ID NO:10 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:9.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB063 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:9.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:9 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:9 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB063 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB063 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB063 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:9 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB065 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB065.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB065 polypeptides comprising a sequence set out in SEQ ID NO:11 which includes a full length gene, or a variant thereof.

The BASB065 polynucleotide provided in SEQ ID NO:11 is the BASB065 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB065 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB065 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB065 polypeptide having a deduced amino acid sequence of SEQ ID NO:12 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB065 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:12 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:11 a polynucleotide of the invention encoding BASB065 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:11 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:11 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:11 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:12 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:11, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 715 of SEQ ID NO:11, encodes the polypeptide of SEQ ID NO:12.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:11 over the entire length of SEQ ID NO:11; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:12 over the entire length of SEQ ID NO:12.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:11 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:11. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., Proc. Natl. Acad. Sci., USA 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB065 polypeptide of SEQ ID NO:12 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 714 of SEQ ID NO:11. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:12. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB065 having an amino acid sequence set out in SEQ ID NO:12. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:12. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB065 variants, that have the amino acid sequence of BASB065 polypeptide of SEQ ID NO:12 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB065 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB065 polypeptide having an amino acid sequence set out in SEQ ID NO:12 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:11.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB065 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:11.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:11 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:11 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB065 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB065 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB065 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:11 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB066 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB066.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB066 polypeptides comprising a sequence set out in SEQ ID NO:13 which includes a full length gene, or a variant thereof.

The BASB066 polynucleotide provided in SEQ ID NO:13 is the BASB066 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB066 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB066 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB066 polypeptide having a deduced amino acid sequence of SEQ ID NO:14 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB066 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:14 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:13 a polynucleotide of the invention encoding BASB066 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:13 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:13 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:13 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:14 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:13, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1174 of SEQ ID NO:13, encodes the polypeptide of SEQ ID NO:14.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:13 over the entire length of SEQ ID NO:13; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:14 over the entire length of SEQ ID NO:14.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:13 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:13. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB066 polypeptide of SEQ ID NO:14 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 1173 of SEQ ID NO:13. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:14. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB066 having an amino acid sequence set out in SEQ ID NO:14. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:14. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB066 variants, that have the amino acid sequence of BASB066 polypeptide of SEQ ID NO:14 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB066 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB066 polypeptide having an amino acid sequence set out in SEQ ID NO:14 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:13.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB066 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:13.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:13 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:13 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB066 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB066 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB066 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:13 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB071 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB071.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB071 polypeptides comprising a sequence set out in SEQ ID NO:15 which includes a full length gene, or a variant thereof.

The BASB071 polynucleotide provided in SEQ ID NO:15 is the BASB071 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB071 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB071 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB071 polypeptide having a deduced amino acid sequence of SEQ ID NO:16 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB071 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:16 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:15 a polynucleotide of the invention encoding BASB071 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing

chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:15 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:15 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:15 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:16 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:15, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 805 of SEQ ID NO:15, encodes the polypeptide of SEQ ID NO:16.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:15 over the entire length of SEQ ID NO:15; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:16 over the entire length of SEQ ID NO:16.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:15 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:15. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz

et al., *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB071 polypeptide of SEQ ID NO:16 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 804 of SEQ ID NO:15. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:16. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB071 having an amino acid sequence set out in SEQ ID NO:16. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:16. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB071 variants, that have the amino acid sequence of BASB071 polypeptide of SEQ ID NO:16 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent

substitutions, additions and deletions, that do not alter the properties and activities of BASB071 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB071 polypeptide having an amino acid sequence set out in SEQ ID NO:16 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:15.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB071 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:15.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA,

followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:15 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:15 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB071 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB071 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB071 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:15 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA 85:* 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NOS:1 – 16 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more

prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff *et al., Hum Mol Genet* (1992) 1: 363, Manthorpe *et al., Hum. Gene Ther.* (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu *et al., J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda *et al., Science* (1989) 243: 375), particle bombardment (Tang *et al., Nature* (1992) 356:152, Eisenbraun *et al., DNA Cell Biol* (1993) 12: 791) and *in vivo* infection using cloned retroviral vectors (Seeger *et al., PNAS USA* (1984) 81: 5849).

Vectors, Host Cells, Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which

are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal cells, such as cells of a yeast, *Kluveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender

expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelian Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella , Shigella, Neisseria, BCG.

These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

Diagnostic, Prognostic, Serotyping and Mutation Assays

This invention is also related to the use of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.

Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished

from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers *et al.*, *Science, 230:* 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton *et al.*, *Proc. Natl. Acad. Sci., USA, 85:* 4397-4401 (1985).

In another embodiment, an array of oligonucleotides probes comprising a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee *et al.*, *Science, 274: 610* (1996)).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO:1,3,5,7,9,11,13,15 or a fragment thereof ;

(b) a nucleotide sequence complementary to that of (a);

(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2,4,6,8,10,12,14,16 or a fragment thereof; or

(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2,4,6,8,10,12,14,16.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among others.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferable, SEQ ID NO:1,3,5,7,9,11,13,15 which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide can be used to identify and analyze mutations.

The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the

polynucleotide sequence. In this way, mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing disease, preferably bacterial infections, more preferably infections caused by *Neisseria meningitidis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of polynucleotide having a sequence of SEQ ID NO:1,3,5,7,9,11,13,15. Increased or decreased expression of a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide can be measured using any on of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

The polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as using hybridization or nucleic acid amplification, using a probe obtained or derived from a bodily sample, to determine the

presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Neisseria meningitidis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1,3,5,7,9,11,13,15 are preferred. Also preferred is a grid comprising a number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2,4,6,8,10,12,14,16.

Antibodies

The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

In certain preferred embodiments of the invention there are provided antibodies against BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature 256:* 495-497 (1975); Kozbor *et al.*, *Immunology Today 4:* 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this

invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti- BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 or from naive libraries (McCafferty, *et al.*, (1990), Nature 348, 552-554; Marks, *et al.*, (1992) *Biotechnology 10*, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature 352:* 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.

Thus, among others, antibodies against BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 -polypeptide or BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 -polynucleotide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complimentarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

Antagonists and Agonists - Assays and Molecules

Polypeptides and polynucleotides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology 1(2):* Chapter 5 (1991).

The screening methods may simply measure the binding of a candidate compound to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide and/or polynucleotide activity in the mixture, and comparing the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB051, BASB057, BASB060, BASB061,

BASB063, BASB065, BASB066 or BASB071 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides or polynucleotides, particularly those compounds that are bacteristatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB051, BASB057, BASB060,

BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 agonists is a competitive assay that combines BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 and a potential agonist with BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 -binding molecules, recombinant BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071

-induced activities, thereby preventing the action or expression of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides and/or polynucleotides by excluding BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem. 56:* 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be

used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 agonists and antagonists, preferably bacteristatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

In a further aspect, the present invention relates to mimotopes of the polypeptide of the invention. A mimotope is a peptide sequence, sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native peptide; or is capable of raising antibodies which recognise the native peptide when coupled to a suitable carrier.

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native polypeptide.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly

Neisseria meningitidis infection. Also provided are methods whereby such immunological response slows bacterial replication.

Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the

form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

In a vaccine composition according to the invention, a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide and/or polynucleotide, or a fragment, or a mimotope, or a variant thereof may be present in a vector, such as the live recombinant vectors described above for example live bacterial vectors.

Also suitable are non-live vectors for the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide, for example bacterial outer-membrane vesicles or "blebs". OM blebs are derived from the outer membrane of the two-layer membrane of Gram-negative bacteria and have been documented in many Gram-negative bacteria (Zhou, L *et al.* 1998. *FEMS Microbiol. Lett.* 163:223-228) including *C. trachomatis* and *C. psittaci.* A non-exhaustive list of bacterial pathogens reported to produce blebs also includes: *Bordetella pertussis, Borrelia burgdorferi, Brucella melitensis, Brucella ovis, Escherichia coli, Haemophilus influenza, Legionella pneumophila,*

Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa and Yersinia enterocolitica.

Blebs have the advantage of providing outer-membrane proteins in their native conformation and are thus particularly useful for vaccines. Blebs can also be improved for vaccine use by engineering the bacterium so as to modify the expression of one or more molecules at the outer membrane. Thus for example the expression of a desired immunogenic protein at the outer membrane, such as the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide, can be introduced or upregulated (e.g. by altering the promoter). Instead or in addition, the expression of outer-membrane molecules which are either not relevant (e.g. unprotective antigens or immunodominant but variable proteins) or detrimental (e.g. toxic molecules such as LPS, or potential inducers of an autoimmune response) can be downregulated. These approaches are discussed in more detail below.

The non-coding flanking regions of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene contains regulatory elements important in the expression of the gene. This regulation takes place both at the transcriptional and translational level. The sequence of these regions, either upstream or downstream of the open reading frame of the gene, can be obtained by DNA sequencing. This sequence information allows the determination of potential regulatory motifs such as the different promoter elements, terminator sequences, inducible sequence elements, repressors, elements responsible for phase variation, the shine-dalgarno sequence, regions with potential secondary structure involved in regulation, as well as other types of regulatory motifs or sequences.

This sequence information allows the modulation of the natural expression of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene. The upregulation of the gene expression may be accomplished by altering the promoter, the shine-dalgarno sequence, potential repressor or operator elements, or any

other elements involved. Likewise, downregulation of expression can be achieved by similar types of modification. Alternatively, by changing phase variation sequences, the expression of the gene can be put under phase variation control, or it may be uncoupled from this regulation. In another approach, the expression of the gene can be put under the control of one or more inducible elements allowing regulated expression. Examples of such regulation include, but are not limited to, induction by temperature shift, addition of inductor substrates like selected carbohydrates or their derivatives, trace elements, vitamins, co-factors, metal ions, etc.

Such modifications as described above can be introduced by several different means. The modification of sequences involved in gene expression can be carried out *in vivo* by random mutagenesis followed by selection for the desired phenotype. Another approach consists in isolating the region of interest and modifying it by random mutagenesis, or site-directed replacement, insertion or deletion mutagenesis. The modified region can then be reintroduced into the bacterial genome by homologous recombination, and the effect on gene expression can be assessed. In another approach, the sequence knowledge of the region of interest can be used to replace or delete all or part of the natural regulatory sequences. In this case, the regulatory region targeted is isolated and modified so as to contain the regulatory elements from another gene, a combination of regulatory elements from different genes, a synthetic regulatory region, or any other regulatory region, or to delete selected parts of the wild-type regulatory sequences. These modified sequences can then be reintroduced into the bacterium via homologous recombination into the genome. A non-exhaustive list of preferred promoters that could be used for up-regulation of gene expression includes the promoters porA, porB, lbpB, tbpB, p110, lst, hpuAB from *N. meningitidis* or *N. gonorroheae;* ompCD, copB, lbpB, ompE, UspA1; UspA2; TbpB from *M. Catarrhalis;* p1, p2, p4, p5, p6, lpD, tbpB, D15, Hia, Hmw1, Hmw2 from *H. influenzae*.

In one example, the expression of the gene can be modulated by exchanging its promoter with a stronger promoter (through isolating the upstream sequence of the gene, in vitro modification of this sequence, and reintroduction into the genome by homologous

recombination). Upregulated expression can be obtained in both the bacterium as well as in the outer membrane vesicles shed (or made) from the bacterium.

In other examples, the described approaches can be used to generate recombinant bacterial strains with improved characteristics for vaccine applications. These can be, but are not limited to, attenuated strains, strains with increased expression of selected antigens, strains with knock-outs (or decreased expression) of genes interfering with the immune response, strains with modulated expression of immunodominant proteins, strains with modulated shedding of outer-membrane vesicles.

Thus, also provided by the invention is a modified upstream region of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene, which modified upstream region contains a heterologous regulatory element which alters the expression level of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 protein located at the outer membrane. The upstream region according to this aspect of the invention includes the sequence upstream of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene. The upstream region starts immediately upstream of the BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 gene and continues usually to a position no more than about 1000 bp upstream of the gene from the ATG start codon. In the case of a gene located in a polycistronic sequence (operon) the upstream region can start immediately preceding the gene of interest, or preceding the first gene in the operon. Preferably, a modified upstream region according to this aspect of the invention contains a heterologous promotor at a position between 500 and 700 bp upstream of the ATG.

Thus, the invention provides a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptide, in a modified bacterial bleb. The invention further provides modified host cells capable of producing the non-live membrane-based bleb vectors. The invention further provides nucleic acid vectors comprising the BASB051,

BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 gene having a modified upstream region containing a heterologous regulatory element.

Further provided by the invention are processes to prepare the host cells and bacterial blebs according to the invention.

Also provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic immunization experiments in animal models of infection with *Neisseria meningitidis*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Neisseria meningitidis* infection, in mammals, particularly humans.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteristatic compounds and solutes which render the formulation isotonic with the

bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

An immune response may be broadly distinguished into two extreme catagories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi

Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).
3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with a carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1μg - 200μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

While the invention has been described with reference to certain BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 polypeptides and polynucleotides, it is to be understood that this covers fragments of the naturally occurring polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

The antigen can also be delivered in the form of whole bacteria (dead or alive) or as subcellular fractions, these possibilities do include *N.meningitidis* itself.

Compositions, kits and administration

In a further aspect of the invention there are provided compositions comprising a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polynucleotide and/or a BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 or BASB071 polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptide discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, solutions, powders and the like.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Sequence Databases, Sequences in a Tangible Medium, and Algorithms

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be

incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GAP program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1):* 387 (1984)), BLASTP, BLASTN (Altschul, S.F. et al., *J. Mol. Biol. 215:* 403-410 (1990), and FASTA(Pearson and Lipman Proc. Natl. Acad. Sci. USA 85; 2444-2448 (1988). The BLAST family of programs is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al., J. Mol. Biol. 215*: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)
Comparison matrix: BLOSSUM62 from Henikoff and Henikoff,
Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)
Gap Penalty: 8
Gap Length Penalty: 2
A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:
Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)
Comparison matrix: matches = +10, mismatch = 0
Gap Penalty: 50
Gap Length Penalty: 3
Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the

reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and • is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleic acid alterations, x_n is the total number of nucleic acids in SEQ ID NO:1, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., • is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n.

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and • is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and $\bullet$ is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural

state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including , for example, upper respiratory tract infection, invasive bacterial diseases, such as bacteremia and meningitis.

EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1

The BASB051 gene in *N.meningitidis* strain ATCC 13090.

The BASB051 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:1. The translation of the BASB051 polynucleotide sequence, shown in SEQ ID NO:2, shows significant similarity to *Neisseria gonorrhoeae* ComL lipoprotein. The BASB051 polypeptide contains a leader sequence characteristic of a lipoprotein signal sequence.

Example 2

The BASB057 gene in *N.meningitidis* strain ATCC 13090.

The BASB057 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:3. The translation of the BASB057 polynucleotide sequence, shown in SEQ ID NO:4, shows significant similarity to *Neisseria gonorrhoeae* MtrE outer membrane lipoprotein. The BASB057 polypeptide conains a leader sequence characteristic of a lipoprotein signal sequence.

Example 3

The BASB060 gene in *N.meningitidis* strain ATCC 13090.

The BASB060 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:5. The translation of the BASB060 polynucleotide sequence, shown in SEQ ID NO:6, shows no significant similarity to any known protein. However, the BASB060 polypeptide contains a leader sequence which is characteristic of a lipoprotein signal sequence and has the characteristics of an outer membrane lipoprotein.

Example 4

The BASB061 gene in *N.meningitidis* strain ATCC 13090.

The BASB061 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:7. The translation of the BASB061 polynucleotide sequence, shown in SEQ ID NO:8, shows significant similarity to *Neisseria meningitidis* mlp gene product. The BASB061 polypeptide contains a leader sequence which is characteristic of a lipoprotein signal sequence and has the characteristics of an outer membrane lipoprotein.

Example 5

The BASB063 gene in *N.meningitidis* strain ATCC 13090.

The BASB063 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:9. The translation of the BASB063 polynucleotide sequence, shown in SEQ ID NO:10, shows significant similarity to any known protein. However, the BASB063 polypeptide does contain a leader sequence which is characteristic of a lipoprotein signal sequence and has the characteristics of an outer membrane lipoprotein.

Example 6

The BASB065 gene in *N.meningitidis* strain ATCC 13090.

The BASB065 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:11. The translation of the BASB065 polynucleotide sequence, shown in SEQ ID NO:12, shows significant similarity to any known protein. However, the BASB065 polypeptide does contain a leader sequence which is characteristic of a lipoprotein signal sequence and has the characteristics of an outer membrane lipoprotein.

Example 7

The BASB066 gene in *N.meningitidis* strain ATCC 13090.

The BASB066 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:13. The translation of the BASB066 polynucleotide sequence, shown in SEQ ID NO:14, shows significant similarity to *Neisseria meningitidis* CtrA protein. The BASB066 polypeptide contains a leader sequence which has the characteristics of a lipoprotein signal sequence and has the characteristics of a protein located in the outer membrane.

Example 8

The BASB071 gene in *N.meningitidis* strain ATCC 13090.

The BASB071 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:15. The translation of the BASB071 polynucleotide sequence, shown in SEQ ID NO:16, shows significant similarity to *Neisseria gonorrhoeae* HisJ protein. The BASB071 polypeptide is contains a leader sequence which has the characteristics of a lipoprotein signal sequence.

Polynucleotide and Polypeptide Sequences

SEQ ID NO:1

Neisseria meningitidis BASB051 polynucleotide sequence from strain ATCC 13090

ATGAAAAAAATTCTTTTAACGGTTTCATTAGGTTTGGCACTGAGTGCCTGTGCCACTCAA
GGTACGGTCGATAAAGATGCTCAGATTACCCAAGATTGGAGTGTGGAGAAGCTCTATGCC
GAAGCCCAGGACGAATTGAACAGCAGCAATTATACGCGGGCTGTCAAGTTATACGAAATC
TTGGAATCGCGCTTCCCCACCAGCCGCCATGCCCGGCAATCCCAACTGGATACCGCATAC
GCCTATTATAAAGACGATGAAAAAGACAAGGCTCTGGCGGCAATCGAACGCTTCCGCCGC
CTCCATCCGCAGCATCCGAATATGGATTACGCGCTGTATCTGCGCGGCTTGGTGCTGTTC
AACGAAGACCAGTCCTTCTTGAACAAACTGGCCTCGCAAGACTGGTCCGACCGCGACCCG
AAAGCCAACCGCGAAGTAACCCAGGCGTTTGCGGAACTCGTCCAACGCTTCCCCAACAGC
AAATACGCCGCCGATGCGACCGCACGCATGGTCAAACTGGTCGATGCACTGGGCGGCAAT
GAAATGTCGGTGGCGCGCTACTACATGAAACGCGGCGCATATATCGCCGCCGCCAACCGC
GCCCAAAAAATTATCGGCAGCTACCAAAATACACGCTATGTCGAAGAATCGCTCGCCATC
TTGGAACTTGCCTACCAAAAACTCGGCAAACCACAGCTTGCCGCCGATACGCGCCGCGTG
TTGGAAACCAACTTCCCGAAAAGCCCGTTTTTGACGCACGCTTGGCAGCCCGACGATATG
CCTTGGTGGCGTTACTGGCATTAA

SEQ ID NO:2

Neisseria meningitidis BASB051 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:1

MKKILLTVSLGLALSACATQGTVDKDAQITQDWSVEKLYAEAQDELNSSNYTRAVKLYEI
LESRFPTSRHARQSQLDTAYAYYKDDEKDKALAAIERFRRLHPQHPNMDYALYLRGLVLF
NEDQSFLNKLASQDWSDRDPKANREVTQAFAELVQRFPNSKYAADATARMVKLVDALGGN
EMSVARYYMKRGAYIAAANRAQKIIGSYQNTRYVEESLAILELAYQKLGKPQLAADTRRV
LETNFPKSPFLTHAWQPDDMPWWRYWH

SEQ ID NO:3

Neisseria meningitidis BASB057 polynucleotide sequence from strain ATCC 13090

ATGGATACTACATTGAAAACCACCTTGACTTCTGTTGCAGCAGCCTTCGCATTATCCGCC
TGCACCATGATTCCCCAATACGAGCAGCCCAAAGTCGAAGTTGCCGAAACGTTTAAAAAC
GATACCGCCGACAGCGGCATCCGTGCGGTCGATTTAGGTTGGCATGACTATTTTGCCGAC
CCGCGCCTGCAAAAGCTGATCGACATCGCACTCGAGCGCAATACCAGTTTGCGTACCGCC
GTATTGAACAGCGAAATCTACCGCAAACAATACATGATTGAGCGCAACAACCTCCTGCCC
ACGCTTGCCGCCAATGCGAACGGCTCGCGCCAAGGCAGCTTGAGCGGCGGCAATGTCAGC
AGCAGCTACAATGTCGGACTGGGTGCGGCATCTTACGAACTCGACCTGTTCGGACGCGTC
CGCAGCAGCAGCGAAGCAGCACTGCAAGGCTATTTTGCAAGTGTCGCCAACCGCGATGCG
GCACATTTGAGCCTGATTGCCACCGTTGCCAAAGCCTATTTCAACGAACGTTATGCCGAA
GAAGCGATGTCTTTGGCGCAGCGTGTTTTGAAAACGCGCGAGGAAACCTACAAGCTGTCC
GAATTACGTTACAAGGCAGGCGTGATTTCCGCCGTCGCCCTACGTCAGCAGGAAGCCCTG
ATCGAATCTGCCAAAGCCGATTATGCCCATGCCGCGCGCAGCCGCGAACAGGCGCGCAAT
GCCTTGGCAACCTTGATTAACCAACCGATACCCGAAGACCTGCCTGCCGGTTTGCCGCTG
GACAAGCAGTTTTTTGTTGAAAAACTGCCGGCCGGTTTGAGTTCCGAAGTATTGCTCGAC
CGTCCCGATATCCGTGCTGCCGAACACGCGCTCAAACAGGCAAACGCCAATATCGGTGCG
GCACGCGCCGCCTTTTTCCCATCCATCCGCCTGACCGGAACCGTCGGTACGGGTTCTGCC

GAATTGGGTGGGTTGTTCAAAAGCGGCACGGGCGTTTGGTCGTTCGCGCCGTCTATTACC
CTGCCGATTTTTACCTGGGGTACGAACAAAGCCAACCTTGATGTAGCCAAGCTGCGCCAA
CAGGCACAAATCGTTGCCTATGAAGCCGCCGTCCAATCCGCATTTCAAGACGTGGCAAAC
GCATTGGCGGCGCGCGAGCAGCTGGATAAAGCCTATGACGCTTTAAGCAAACAAAGCCGC
GCCTCTAAAGAGGCGTTGCGCTTGGTCGGCCTGCGTTACAAGCACGGCGTATCCGGCGCG
CTCGACTTGCTCGATGCGGAACGCAGCAGCTATGCGGCGGAGGGTGCGGCTTTGTCGGCA
CAACTGACCCGCGCCGAAAACCTTGCCGATTTGTACAAGGCACTCGGCGGCGGATTGAAA
CGGGATACCCAAACCGACAAATAA

SEQ ID NO:4

Neisseria meningitidis BASB057 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:3

MDTTLKTTLTSVAAAFALSACTMIPQYEQPKVEVAETFKNDTADSGIRAVDLGWHDYFAD
PRLQKLIDIALERNTSLRTAVLNSEIYRKQYMIERNNLLPTLAANANGSRQGSLSGGNVS
SSYNVGLGAASYELDLFGRVRSSSEAALQGYFASVANRDAAHLSLIATVAKAYFNERYAE
EAMSLAQRVLKTREETYKLSELRYKAGVISAVALRQQEALIESAKADYAHAARSREQARN
ALATLINQPIPEDLPAGLPLDKQFFVEKLPAGLSSEVLLDRPDIRAAEHALKQANANIGA
ARAAFFPSIRLTGTVGTGSAELGGLFKSGTGVWSFAPSITLPIFTWGTNKANLDVAKLRQ
QAQIVAYEAAVQSAFQDVANALAAREQLDKAYDALSKQSRASKEALRLVGLRYKHGVSGA
LDLLDAERSSYAAEGAALSAQLTRAENLADLYKALGGGLKRDTQTDK

SEQ ID NO:5

Neisseria meningitidis BASB060 polynucleotide sequence from strain ATCC 13090

ATGAAAAAACTTCTAATGATAACCCTCACCGGTATGCTTGCAGCTTGTGCAACAGGTGTC
AATGTCGGCCGGTTGATGGTTGAAATGCCGCAGGGAGAACGTTCTGTCGTTGTGCAGGTT
CCCGCGACAAATAACCCGCTTTCCGATACGGTAGCTGTCGGAATGATTAAAACATCCGGT
TCGCCTTCGGCATCAAATATGATTGAAATGCTCGGCGCGGACAATATCAACGTCGGCGTG
GTGGGAAGCAGCCAAATGCTTAATAAGGCGACCGCACTTTATTCCTTAAACCATGCAAAG
AAAGTCGGAAATAATGTCAGTGTTTATATGATGGGCGACAGCGAAAGTGACAAGGCCGAT
TTGGAAAACGCGGCAAATGCCAAAAATATCAAATTGCATTATTTCTTTAACCAAAAATAA

SEQ ID NO:6

Neisseria meningitidis BASB060 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:5

MKKLLMITLTGMLAACATGVNVGRLMVEMPQGERSVVVQVPATNNPLSDTVAVGMIKTSG
SPSASNMIEMLGADNINVGVVGSSQMLNKATALYSLNHAKKVGNNVSVYMMGDSESDKAD
LENAANAKNIKLHYFFNQK

SEQ ID NO:7

Neisseria meningitidis BASB061 polynucleotide sequence from strain ATCC 13090

ATGAAAATCAAACAAATCGTCAAACCGGGCTTGGCAGTATTGGCGGCGGGCGTTCTGTCT
GCCTGCGCAACCAAAAGCAACGTCAAAGCCGACGGAACGACCGACAATCCGGTTTTCCCG
AAACCCTATTCCGTAACGCTCGACAACAATCGCGGTACATTCCCGACCTATGACGAATTG

GACTTGATGCGTCCCGGTCTGACCAAAGACGACATCTACAAAATCCTGGGTCGTCCGCAT
TACGACGAAGGTATGTACGGCGTGCGCGAATGGGATTATCTGTTCCACTTCCACACCCCG
GGCGTAGGCATCGACCCTGAAAACACTTCCGGCGTAGAAGGCATTACCACCTGTCAATAC
AAAATTATTTTCGATAAAGACAAATTTGCCCGCAGCTTCTACTGGAACCCCGTCTTCCCG
AAAGATGCCGCCTGTCCGCCGCCCGCACCCAAAGCCGAGCCGCAAgTCATCATCCGCGAA
ATCGTGCCCGCCAAAcCCAAACGCATCCGCCAATAA

SEQ ID NO:8

Neisseria meningitidis BASB061 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:7

MKIKQIVKPGLAVLAAGVLSACATKSNVKADGTTDNPVFPKPYSVTLDNNRGTFPTYDEL
DLMRPGLTKDDIYKILGRPHYDEGMYGVREWDYLFHFHTPGVGIDPENTSGVEGITTCQY
KIIFDKDKFARSFYWNPVFPKDAACPPPAPKAEPQVIIREIVPAKPKRIRQ

SEQ ID NO:9

Neisseria meningitidis BASB063 polynucleotide sequence from strain ATCC 13090

ATGAGACCATATGCTACTACCATTTATCAACTTTTTATTTTGTTTATTGGGAGTGTTTTTT
ACTATGACCTCATGTGAACCTGTGAATGAAAAGACAGATCAAAAAGCAGTAAGTGCGCAA
CAGGCTAAAGAACAAACCAGTTTCAACAATCCCGAGCCAATGACAGGATTTGAACATACG
GTTACATTTGATTTTCAGGGCACCAAAATGGTTATCCCCTATGGCTATCTTGCACGGTAT
ACGCAAGACAATGCCACAAAATGGCTTTCCGACACGCCCGGGCAGGATGCTTACTCCATT
AATTTGATAGAGATTAGCGTCTATTACAAAAAAACCGACCAAGGCTGGGTTCTTGAGCCA
TACAACCAGCAAAACAAAGCACACTTTATCCAATTTCTACGCGACGGTTTGGATAGCGTG
GACGATATTGTTATCCGAAAAGATGCGTGTAGTTTAAGTACGACTATGGGAGAAAGATTG
CTTACTTACGGGGTTAAAAAAATGCCATCTGCCTATCCTGAATACGAGGCTTATGAAGAT
AAAAGACATATTCCTGAAAATCCATATTTTCATGAATTTTACTATATTAAAAAAGGAGAA
AATCCGGCGATTATTACTCATCGGAATAATCGAATAAACCAAACTGAAGAAGATAGTTAT
AGCACTAGCGTAGGTTCCTGTATTAACGGTTTCACGGTACAGTATTACCCGTTTATTCGG
GAAAAGCAGCAGCTCACACAGCAGGAGTTGGTAGGTTATCACCAACAAGTAGAGCAATTG
GTACAGAGTTTTGTAAACAATTCAAATAAAAAATAA

SEQ ID NO:10

Neisseria meningitidis BASB063 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:9

MRPYATTIYQLFILFIGSVFTMTSCEPVNEKTDQKAVSAQQAKEQTSFNNPEPMTGFEHT
VTFDFQGTKMVIPYGYLARYTQDNATKWLSDTPGQDAYSINLIEISVYYKKTDQGWVLEP
YNQQNKAHFIQFLRDGLDSVDDIVIRKDACSLSTTMGERLLTYGVKKMPSAYPEYEAYED
KRHIPENPYFHEFYYIKKGENPAIITHRNNRINQTEEDSYSTSVGSCINGFTVQYYPFIR
EKQQLTQQELVGYHQQVEQLVQSFVNNSNKK

SEQ ID NO:11

Neisseria meningitidis BASB065 polynucleotide sequence from strain ATCC 13090

ATGAAGACCAAATTACCGCTTTTTATCATTTGGCTGTCCGTATCCGCCGCCTGTTCTTCC

CCTGTTTCCCGCAATATTCAGGATATGCGGCCCGAACCGCAGGCAGAGGCAGGTAGTTCG
GACGCTATTCCCTATCCCGTTCCCACTCTGCAAGACCGTTTGGATTATCTGGAAGGCACA
CTCGTCCGCCTGTCGAACGAAGTGGAAACCTTAAACGGCAAAGTCAAAGCACTGGAGCAT
GCGAAAACACACCCTTCCGGTAGGGCATACGTCCAAAAACTCGACGACCGCAAGTTGAAA
GAGCATTACCTCAATACCGAAGGCGGCAGCGCATCCGCACATACCGTCGAAACCGCACAA
AACCTCTACAATCAGGCACTCAAACACTATAAAAGCGGCAGGTTTTCTGCCGCAGCCGCC
CTGTTGAAAGGCGCGGACGGAGGCGACGGCGGCAGCATCGCGCAACGCAGTATGTACCTG
TTGCTGCAAAGCAGGGCGCGTATGGGCAACTGCGAATCCGTCATCGAAATCGGAGGGCGT
TACGCCAACCGTTTCAAAGACAGCCCAACCGCGCCCGAAGCCATGTTCAAAATCGGCGAA
TGCCAATACAGGTTGCAGCAGAAAGACATTGCAAGGGCAACTTGGCGCAGCCTGATACAG
GCTTACCCGAGCAGCCCGGCGGCAAAACGCGCCGCCGCAGCCGTACGCAAACGATAG

SEQ ID NO:12

Neisseria meningitidis BASB065 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:11

MKTKLPLFIIWLSVSAACSSPVSRNIQDMRPEPQAEAGSSDAIPYPVPTLQDRLDYLEGT
LVRLSNEVETLNGKVKALEHAKTHPSGRAYVQKLDDRKLKEHYLNTEGGSASAHTVETAQ
NLYNQALKHYKSGRFSAAAALLKGADGGDGGSIAQRSMYLLLQSRARMGNCESVIEIGGR
YANRFKDSPTAPEAMFKIGECQYRLQQKDIARATWRSLIQAYPSSPAAKRAAAAVRKR

SEQ ID NO:13

Neisseria meningitidis BASB066 polynucleotide sequence from strain ATCC 13090

GTGTTTAAAGTGAAATTTTATATTCGTCACGCAGTATTATTATTGTGTGGAAGTTTAATT
GTAGGATGCTCTGCGATTCCTTCATCAGGCCCCAGCGCAAAAAAAATTGTCTCTTTAGGG
CAACAATCTGAAGTTCAAATTCCTGAAGTGGAGCTGATTGATGTGAATCATACGGTTGCT
CAGTTATTATATAAGGCTCAGATAAATCAGTCATTCACTCAGTTTGGCGATGGTTATGCT
TCGGCTGGTACGCTAAATATTGGTGATGTATTGGATATTATGATTTGGGAAGCGCCGCCG
GCAGTATTGTTTGGTGGTGGCCTTTCTTCGATGGGCTCGGGTAGTGCGCATCAAACTAAG
TTGCCAGAGCAGTTGGTCACGGCACGTGGTACGGTTTCTGTGCCGTTTGTTGGCGATATT
TCGGTGGTCGGTAAAACGCCTGGTCAGGTTCAGGAAATTATTAAAGGCCGCCTGAAAAAA
ATGGCCAATCAGCCACAAGTGATGGTGCGTTTGGTGCAGAATAATGCGGCGAATGTGTCG
GTGATTCGTGCTGGGAATAGTGTGCGTATGCCGCTGACGGCAGCCGGTGAGCGTGTGTTG
GATGCGGTGGCTGCGGTAGGTGGTTCAACGGCAAATGTGCAGGATACGAATGTGCAGCTG
ACACGTGGCAATGTAGTACGAACTGTTGCCTTGGAAGATTTAGTTGCAAATCCGCGACAA
AATATTTTGCTGCGTCGCGGTGATGTGGTTACCATGATTACCAATCCCTATACCTTTACG
TCTATGGGTGCGGTGGGGAGAACACAAGAAATCGGTTTTTCAGCCAGAGGCTTATCGCTT
TCTGAAGCCATTGGCCGTATGGGCGGTTTGCAAGATCGCCGTTCTGATGCGCGTGGTGTG
TTTGTGTTCCGCTATACGCCATTGGTGGAATTGCCGGCAGAACGTCAGGATAAATGGATT
GCTCAAGGTTATGGCAGTGAGGCAGAGATTCCAACGGTATATCGTGTGAATATGGCTGAT
GCGCATTCGCTATTTTCTATGCAGCGCTTTCCTGTGAAGAATAAAGATGTATTGTATGTG
TCGAATGCGCCGTTGGCTGAAGTGCAGAAATTCTTGTCGTTTGTGTTCTCGCCGGTTACC
AGTGGCGCGAACAGTATTAATAATTTAACTAATTAA

SEQ ID NO:14

Neisseria meningitidis BASB066 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:13

MFKVKFYIRHAVLLLCGSLIVGCSAIPSSGPSAKKIVSLGQQSEVQIPEVELIDVNHTVA
QLLYKAQINQSFTQFGDGYASAGTLNIGDVLDIMIWEAPPAVLFGGGLSSMGSGSAHQTK
LPEQLVTARGTVSVPFVGDISVVGKTPGQVQEIIKGRLKKMANQPQVMVRLVQNNAANVS
VIRAGNSVRMPLTAAGERVLDAVAAVGGSTANVQDTNVQLTRGNVVRTVALEDLVANPRQ
NILLRRGDVVTMITNPYTFTSMGAVGRTQEIGFSARGLSLSEAIGRMGGLQDRRSDARGV
FVFRYTPLVELPAERQDKWIAQGYGSEAEIPTVYRVNMADAHSLFSMQRFPVKNKDVLYV
SNAPLAEVQKFLSFVFSPVTSGANSINNLTN

SEQ ID NO:15

Neisseria meningitidis BASB071 polynucleotide sequence from strain ATCC 13090

ATGAATATGAAAAAATGGATTGCCGCCGCCCTTGCCTGTTCCGCGCTCGCGCTGTCTGCC
TGCGGCGGTCAGGGCAAAGATGCCGCCGCGCCCGCCGCAAACCCCGACAAAGTGTACCGC
GTGGCTTCCAACGCCGAGTTTGCCCCCTTTGAATCTTTAGACTCGAAAGGCAATGTTGAA
GGTTTCGATGTGGATTTGATGAACGCGATGGCGAAGGCGGGCAATTTTAAAATCGAATTC
AAACACCAGCCGTGGGACAGCCTTTTCCCCGCCTTGAACAACGGCGATGCGGACGTTGTG
ATGTCGGGCGTAACCATTACCGACGACCGCAAACAGTCTATGGACTTCAGCGACCCGTAT
TTTGAAATCACCCAAGTCGTCCTCGTTCCGAAAGGCAAAAAAATATCTTCTTCCGAAGAT
TTGAAAAACATGAACAAAGTCGGCGTGGTAACCGGCTACACGGGCGATTTCTCCGTATCC
AAACTCTTGGGCAACGACAACCCGAAAATCGCGCGCTTTGAAAACGTTCCCCTGATTATC
AAAGAACTGGAAAACGGCGGCTTGGATTCCGTGGTCAGCGACAGCGCAGTCATCGCCAAT
TATGTGAAAAACAATCCGACCAAAGGGATGGACTTCGTTACCCTGCCCGACTTCACCACC
GAACACTACGGCATCGCGGTACGCAAAGGCGACGAAGCAACCGTCAAAATGCTGAACGAT
GCGTTGAAAAAAGTACGCGAAAGCGGCGAATACGACAAAATCTACGCCAAATATTTTGCA
AAAGAAGACGGACAGGCCGCAAAATAA

SEQ ID NO:16

Neisseria meningitidis BASB071 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:15

MNMKKWIAAALACSALALSACGGQGKDAAAPAANPDKVYRVASNAEFAPFESLDSKGNVE
GFDVDLMNAMAKAGNFKIEFKHQPWDSLFPALNNGDADVVMSGVTITDDRKQSMDFSDPY
FEITQVVLVPKGKKISSSEDLKNMNKVGVVTGYTGDFSVSKLLGNDNPKIARFENVPLII
KELENGGLDSVVSDSAVIANYVKNNPTKGMDFVTLPDFTTEHYGIAVRKGDEATVKMLND
ALKKVRESGEYDKIYAKYFAKEDGQAAK

Deposited materials

A deposit containing a *Neisseria meningitidis* Serogroup B strain has been deposited with the American Type Culture Collection (herein "ATCC") on June 22, 1997 and assigned deposit number 13090. The deposit was described as *Neisseria meningitidis* (Albrecht and Ghon) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *N. meningitidis* isolate. The deposit is described in Int. Bull. Bacteriol. Nomencl. Taxon. 8: 1-15 (1958).

The *Neisseria meningitidis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full-length BASB051, BASB057, BASB060, BASB061, BASB063, BASB065, BASB066 and BASB071 genes. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

Applicant's or agent's file reference	International application No.
KP/BM45348	

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 110, line 2-22

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

10801 UNIVERSITY BLVD, MANASSAS, VIRGINIA 20110-2209,
UNITED STATES OF AMERICA

Date of deposit	Accession Number
22 June 1997 (22.06.97)	13090

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of those designations where a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European Patent or until the date on which the application has been refused or withdrawn, only by issue of such a sample to an expert nominated by the person requesting the sample.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

CLAIMS

1. An isolated polypeptide comprising an amino acid sequence which has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.

4. An isolated polypeptide of : SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16.

5. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 4 in which the immunogenic activity of said immunogenic fragment is substantially the same as that of the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16.

6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 85% identity to an amino acid sequence of SEQ ID NO:2,4,6,8,10,12,14 or 16 over the entire length of SEQ ID NO:2,4,6,8,10,12,14 or 16 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

7. An isolated polynucleotide comprising a nucleotide sequence that has at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2,4,6,8,10,12,14 or 16 over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 85% identity to that of SEQ ID NO:1,3,5,7,9,11,13 or 15 over the entire length of SEQ ID NO:1,3,5,7,9,11,13 or 15 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:1,3,5,7,9,11,13 or 15.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16.

11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 or SEQ ID NO:15.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16 obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 or SEQ ID NO:15 or a fragment thereof.

13. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 to 12.

14. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16.

15. A process for producing a polypeptide comprising an amino acid sequence that has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16 comprising culturing a host cell of claim 14 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

16. A process for expressing a polynucleotide of any one of claims 6 to 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

17. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

18. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically effective carrier.

19. The vaccine composition according to either one of claims 17 or 18 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.

20. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

21. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 to 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

22. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 to 5 in the preparation of a medicament for use in generating an immune response in an animal.

23. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 to 12 in the preparation of a medicament for use in generating an immune response in an animal.

24. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 to 5 and a suitable pharmaceutical carrier.

SEQUENCE LISTING

<110> SmithKline Beecham Biologicals S.A.

<120> Novel compounds

<130> BM45348

<160> 16

<170> FastSEQ for Windows Version 3.0

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Trp Ser Val Glu Lys Leu Tyr Ala Glu Ala Gln Asp Glu Leu Asn Ser
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        35                  40                  45
Ser Asn Tyr Thr Arg Ala Val Lys Leu Tyr Glu Ile Leu Glu Ser Arg
    50                  55                  60
Phe Pro Thr Ser Arg His Ala Arg Gln Ser Gln Leu Asp Thr Ala Tyr
65                  70                  75                  80
Ala Tyr Tyr Lys Asp Asp Glu Lys Asp Lys Ala Leu Ala Ala Ile Glu
                85                  90                  95
Arg Phe Arg Arg Leu His Pro Gln His Pro Asn Met Asp Tyr Ala Leu
            100                 105                 110
Tyr Leu Arg Gly Leu Val Leu Phe Asn Glu Asp Gln Ser Phe Leu Asn
        115                 120                 125
Lys Leu Ala Ser Gln Asp Trp Ser Asp Arg Asp Pro Lys Ala Asn Arg
    130                 135                 140
Glu Val Thr Gln Ala Phe Ala Glu Leu Val Gln Arg Phe Pro Asn Ser
145                 150                 155                 160
Lys Tyr Ala Ala Asp Ala Thr Ala Arg Met Val Lys Leu Val Asp Ala
                165                 170                 175
Leu Gly Gly Asn Glu Met Ser Val Ala Arg Tyr Tyr Met Lys Arg Gly
            180                 185                 190
Ala Tyr Ile Ala Ala Ala Asn Arg Ala Gln Lys Ile Ile Gly Ser Tyr
        195                 200                 205
Gln Asn Thr Arg Tyr Val Glu Glu Ser Leu Ala Ile Leu Glu Leu Ala
    210                 215                 220
Tyr Gln Lys Leu Gly Lys Pro Gln Leu Ala Ala Asp Thr Arg Arg Val
225                 230                 235                 240
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gaattacgtt acaaggcagg cgtgatttcc gccgtcgccc tacgtcagca ggaagccctg     660
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<400> 4

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35 40 45
Ala Val Asp Leu Gly Trp His Asp Tyr Phe Ala Asp Pro Arg Leu Gln
50 55 60
Lys Leu Ile Asp Ile Ala Leu Glu Arg Asn Thr Ser Leu Arg Thr Ala
65 70 75 80
Val Leu Asn Ser Glu Ile Tyr Arg Lys Gln Tyr Met Ile Glu Arg Asn
85 90 95
Asn Leu Leu Pro Thr Leu Ala Ala Asn Ala Asn Gly Ser Arg Gln Gly
100 105 110
Ser Leu Ser Gly Gly Asn Val Ser Ser Ser Tyr Asn Val Gly Leu Gly
115 120 125
Ala Ala Ser Tyr Glu Leu Asp Leu Phe Gly Arg Val Arg Ser Ser Ser
130 135 140
Glu Ala Ala Leu Gln Gly Tyr Phe Ala Ser Val Ala Asn Arg Asp Ala
145 150 155 160
Ala His Leu Ser Leu Ile Ala Thr Val Ala Lys Ala Tyr Phe Asn Glu
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Arg Tyr Ala Glu Glu Ala Met Ser Leu Ala Gln Arg Val Leu Lys Thr
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                245                 250                 255
Gly Leu Pro Leu Asp Lys Gln Phe Phe Val Glu Lys Leu Pro Ala Gly
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Leu Ser Ser Glu Val Leu Leu Asp Arg Pro Asp Ile Arg Ala Ala Glu
        275                 280                 285
His Ala Leu Lys Gln Ala Asn Ala Asn Ile Gly Ala Ala Arg Ala Ala
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Phe Phe Pro Ser Ile Arg Leu Thr Gly Thr Val Gly Thr Gly Ser Ala
305                 310                 315                 320
Glu Leu Gly Gly Leu Phe Lys Ser Gly Thr Gly Val Trp Ser Phe Ala
                325                 330                 335
Pro Ser Ile Thr Leu Pro Ile Phe Thr Trp Gly Thr Asn Lys Ala Asn
            340                 345                 350
Leu Asp Val Ala Lys Leu Arg Gln Gln Ala Gln Ile Val Ala Tyr Glu
        355                 360                 365
Ala Ala Val Gln Ser Ala Phe Gln Asp Val Ala Asn Ala Leu Ala Ala
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Arg Glu Gln Leu Asp Lys Ala Tyr Asp Ala Leu Ser Lys Gln Ser Arg
385                 390                 395                 400
Ala Ser Lys Glu Ala Leu Arg Leu Val Gly Leu Arg Tyr Lys His Gly
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Val Ser Gly Ala Leu Asp Leu Leu Asp Ala Glu Arg Ser Ser Tyr Ala
            420                 425                 430
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tcgccttcgg catcaaatat gattgaaatg ctcggcgcgg acaatatcaa cgtcggcgtg      240
gtgggaagca gccaaatgct taataaggcg accgcacttt attccttaaa ccatgcaaag      300
aaagtcggaa ataatgtcag tgtttatatg atgggcgaca gcgaaagtga caaggccgat      360
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<210> 6
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<213> *Neisseria meningitidis*

<400> 6

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            20                  25                  30
Glu Arg Ser Val Val Val Gln Val Pro Ala Thr Asn Asn Pro Leu Ser
        35                  40                  45
Asp Thr Val Ala Val Gly Met Ile Lys Thr Ser Gly Ser Pro Ser Ala
    50                  55                  60
Ser Asn Met Ile Glu Met Leu Gly Ala Asp Asn Ile Asn Val Gly Val
65                  70                  75                  80
Val Gly Ser Ser Gln Met Leu Asn Lys Ala Thr Ala Leu Tyr Ser Leu
                85                  90                  95
Asn His Ala Lys Lys Val Gly Asn Asn Val Ser Val Tyr Met Met Gly
            100                 105                 110
Asp Ser Glu Ser Asp Lys Ala Asp Leu Glu Asn Ala Ala Asn Ala Lys
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35 40 45
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65 70 75 80
Tyr Asp Glu Gly Met Tyr Gly Val Arg Glu Trp Asp Tyr Leu Phe His
85 90 95
Phe His Thr Pro Gly Val Gly Ile Asp Pro Glu Asn Thr Ser Gly Val
100 105 110
Glu Gly Ile Thr Thr Cys Gln Tyr Lys Ile Ile Phe Asp Lys Asp Lys
115 120 125
Phe Ala Arg Ser Phe Tyr Trp Asn Pro Val Phe Pro Lys Asp Ala Ala
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Asp Gln Lys Ala Val Ser Ala Gln Gln Ala Lys Glu Gln Thr Ser Phe
        35                  40                  45
Asn Asn Pro Glu Pro Met Thr Gly Phe Glu His Thr Val Thr Phe Asp
    50                  55                  60
Phe Gln Gly Thr Lys Met Val Ile Pro Tyr Gly Tyr Leu Ala Arg Tyr
65                  70                  75                  80
Thr Gln Asp Asn Ala Thr Lys Trp Leu Ser Asp Thr Pro Gly Gln Asp
                85                  90                  95
Ala Tyr Ser Ile Asn Leu Ile Glu Ile Ser Val Tyr Tyr Lys Lys Thr
            100                 105                 110
Asp Gln Gly Trp Val Leu Glu Pro Tyr Asn Gln Gln Asn Lys Ala His
        115                 120                 125
Phe Ile Gln Phe Leu Arg Asp Gly Leu Asp Ser Val Asp Asp Ile Val
    130                 135                 140
Ile Arg Lys Asp Ala Cys Ser Leu Ser Thr Thr Met Gly Glu Arg Leu
145                 150                 155                 160
Leu Thr Tyr Gly Val Lys Lys Met Pro Ser Ala Tyr Pro Glu Tyr Glu
                165                 170                 175
Ala Tyr Glu Asp Lys Arg His Ile Pro Glu Asn Pro Tyr Phe His Glu
            180                 185                 190
Phe Tyr Tyr Ile Lys Lys Gly Glu Asn Pro Ala Ile Ile Thr His Arg
        195                 200                 205
Asn Asn Arg Ile Asn Gln Thr Glu Glu Asp Ser Tyr Ser Thr Ser Val
    210                 215                 220
Gly Ser Cys Ile Asn Gly Phe Thr Val Gln Tyr Tyr Pro Phe Ile Arg
225                 230                 235                 240
Glu Lys Gln Gln Leu Thr Gln Gln Glu Leu Val Gly Tyr His Gln Gln
                245                 250                 255
Val Glu Gln Leu Val Gln Ser Phe Val Asn Asn Ser Asn Lys Lys
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            20                  25                  30
Pro Gln Ala Glu Ala Gly Ser Ser Asp Ala Ile Pro Tyr Pro Val Pro
        35                  40                  45
Thr Leu Gln Asp Arg Leu Asp Tyr Leu Glu Gly Thr Leu Val Arg Leu
    50                  55                  60
Ser Asn Glu Val Glu Thr Leu Asn Gly Lys Val Lys Ala Leu Glu His
65                  70                  75                  80
Ala Lys Thr His Pro Ser Gly Arg Ala Tyr Val Gln Lys Leu Asp Asp
                85                  90                  95
Arg Lys Leu Lys Glu His Tyr Leu Asn Thr Glu Gly Gly Ser Ala Ser
            100                 105                 110
Ala His Thr Val Glu Thr Ala Gln Asn Leu Tyr Asn Gln Ala Leu Lys
        115                 120                 125
His Tyr Lys Ser Gly Arg Phe Ser Ala Ala Ala Ala Leu Leu Lys Gly
    130                 135                 140
Ala Asp Gly Gly Asp Gly Gly Ser Ile Ala Gln Arg Ser Met Tyr Leu
145                 150                 155                 160
Leu Leu Gln Ser Arg Ala Arg Met Gly Asn Cys Glu Ser Val Ile Glu
                165                 170                 175
Ile Gly Gly Arg Tyr Ala Asn Arg Phe Lys Asp Ser Pro Thr Ala Pro
            180                 185                 190
Glu Ala Met Phe Lys Ile Gly Glu Cys Gln Tyr Arg Leu Gln Gln Lys
        195                 200                 205
Asp Ile Ala Arg Ala Thr Trp Arg Ser Leu Ile Gln Ala Tyr Pro Ser
    210                 215                 220
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225                 230                 235
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<400> 14

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            20                  25                  30
Ala Lys Lys Ile Val Ser Leu Gly Gln Gln Ser Glu Val Gln Ile Pro
        35                  40                  45
Glu Val Glu Leu Ile Asp Val Asn His Thr Val Ala Gln Leu Leu Tyr
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Pro Phe Glu Ser Leu Asp Ser Lys Gly Asn Val Glu Gly Phe Asp Val
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Asp Leu Met Asn Ala Met Ala Lys Ala Gly Asn Phe Lys Ile Glu Phe
65                  70                  75                  80
Lys His Gln Pro Trp Asp Ser Leu Phe Pro Ala Leu Asn Asn Gly Asp
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Ser Met Asp Phe Ser Asp Pro Tyr Phe Glu Ile Thr Gln Val Val Leu
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Val Pro Lys Gly Lys Lys Ile Ser Ser Ser Glu Asp Leu Lys Asn Met
    130                 135                 140
Asn Lys Val Gly Val Val Thr Gly Tyr Thr Gly Asp Phe Ser Val Ser
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Lys Leu Leu Gly Asn Asp Asn Pro Lys Ile Ala Arg Phe Glu Asn Val
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Pro Leu Ile Ile Lys Glu Leu Glu Asn Gly Gly Leu Asp Ser Val Val
            180                 185                 190
Ser Asp Ser Ala Val Ile Ala Asn Tyr Val Lys Asn Asn Pro Thr Lys
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```

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245 250 255

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260 265

D

09/889283

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70) 14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
KP/BM45348		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00135	10/01/2000	15/01/1999

International Patent Classification (IPC) or national classification and IPC
C12N15/31

Applicant
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01/08/2000	09.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Chavanne, F Telephone No. +49 89 2399 8399

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00135

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-110 as originally filed

Claims, No.:

1-25 as received on 02/03/2001 with letter of 01/03/2001

Sequence listing part of the description, pages:

1-12, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00135

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-25 partially.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-25 partially.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-5, 13-25
	No:	Claims 6-12
Inventive step (IS)	Yes:	Claims

	No:	Claims	1-25
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The subject-matter of claims 1-25 has been partially searched. Consequently, an opinion with regard to novelty, inventive step and industrial applicability can only be partially formulated, and limited to the invention referred to as invention 1 in the international search report, namely:
Invention 1: polypeptides comprising a sequence homologous to SEQ ID NO:2, immunogenic fragments thereof, polynucleotides encoding said polypeptides, process for their production, vaccines containing them, antibodies specific for said polypeptides, as well as diagnostic and pharmaceutical uses thereof.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: Molecular Microbiology
Vol. 19, No. 5, pp. 1095-1105, 1996
D2: WO 98/02547

3. The scope of claims 6-12 encompasses a nucleic acid molecule comprising the sequence of SEQ ID No. 1. Because of the expression "comprising", said nucleic acid molecule is not necessarily limited to the sequence of SEQ ID No.1, but may also contain other sequences. The genomic DNA of *Neisseria meningitidis*, which has already been isolated (see e.g. D2), is a DNA molecule which comprises the sequence of SEQ ID No.1, and thus, falls within the scope of claims 6-12. Thus, due to the too broad formulation of claims 6-12, the subject-matter of these claims is not novel.
Therefore, claims 6-12 do not meet the requirements of Article 33(2) PCT.

4. The closest prior art to evaluate the inventiveness of the present application is D1. D1 describes a polypeptide from *Neisseria gonorrhoeae* with an amino acid

sequence showing over 97.75% identity with the amino acid sequence of SEQ ID NO:2. The nucleotide sequence of the *comL* gene which encodes said polypeptide is also disclosed, and has been cloned into an expression vector for the specific expression of said polypeptide (abstract; Figures 2-5). Moreover, D1 shows that the homologous counterpart of the *comL* gene is present in *Neisseria meningitidis* and that both genes are highly homologous (abstract; page 1100, column 2, paragraph 3; figure 6).

The subject-matter of claims 1-4 differs from D1 in that the polypeptide of SEQ ID NO. 2 and the isolated polypeptide disclosed in D1 do not have the exact same amino acid sequence. They correspond to homologous products from different *Neisseria* species. However, knowing the *comL* gene from *N. gonorrhoeae*, and being aware of the presence of the *comL* gene highly homologous counterpart in *N. meningitidis*, the man skilled in the art would not require any inventive skill to isolate said gene, and by further applying commonly used technics would automatically come to the subject-matter of claims 1-4 and 6-17. Thus, said subject-matter is not inventive.

The subject-matter of claim 5 further differs from D1 in that D1 does not mention any fragment of the disclosed polypeptide. However, any polypeptide or fragment of a known polypeptide is immunogenic since it triggers an immune response in animals. Thus, the subjcet-matter of claim 5 is not inventive.

The present application does not describe the immunological activity of the claimed polypeptide. However, with the assumption that said polypeptide induces an immunological response, the man skilled in the art, by further applying common knowledge would also come to the subject-matter of claims 18-25. Thus, said subject-matter is not inventive.

Therefore, claims 1-25 do not meet the requirements of Article 33(3) PCT.

VII. Certain defects in the international application

1. The present application does not contain any example showing the best mode for carrying out the invention as required by Rule 5.1(v) (see also Guidelines C-II, 4.9).

2. Although claims 10 and 12 have been drafted as separate independent claims, they both relate to an isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID No.2. The same subject-matter of these claims only differs from each other only in that claim 12 tries to further define said isolated polynucleotide in that it is obtainable by screening with a probe having the sequence of SEQ ID No.1. It appears appropriate to amend said claims by defining the relevant subject-matter in terms of one single independent claim followed by dependent claims covering the optional features (Rule 6.4 PCT).

VIII. Certain observations on the international application

1. The formulation "...comprising..." or "..which comprises..." in claims 6-8 and 10-12 does not clearly define the scope of the claim. Thus, this expression should be replaced with "consisting of" or "which consists of", respectively (Article 6 PCT).

2. Claim 5 refers to an immunogenic fragment capable of raising an immune response which recognises the polypeptide of SEQ ID No. 2. However, the present application does not describe any immunogenic fragment of any polypeptide. Therefore, the subject-matter of claim 5 is not based on the description (Art. 6-support PCT). Since the description does not mention any immunogenic fragment, the man skilled in the art, faced with the problem of providing such a fragment capable of raising an immune response which recognises the polypeptide of SEQ ID No.2, would not be able to provide it without the need of applying intensive experimentations of undue burden. Therefore, with regard to claim 5, the present application does not meet the requirements of Article 5 PCT.
   Moreover, it should be noted that formulations in parentheses are merely optional, and thus, not adapted to limit the scope of the claim.

3. The subject-matter of claims 18-20 refers to a vaccine composition comprising the claimed polypeptide. However, apart from vague declarations on pages 81-92, the specification of the present application neither describes a vaccine composition nor shows that the claimed polypeptide would be effective for a vaccine composition. Moreover, the present application do not provide any evidence that

the claimed polypeptide is adapted for the preparation of immunospecific antibodies, or that the claimed polypeptide can be immunologically effective. Thus, the subject-matter of claims 18-25 is not based on the description (Art. 6-support PCT).

Moreover, with the assumption that the polypeptide described in the present application has the same internal cellular localisation as its homologous counterpart from *N. gonorrhoeae* (see D1, page 1100, column 1, paragraph 2), it is not clear how the immunological characteristics of said polypeptide may be adapted for the preparation of e.g. therapeutical compositions, for its use in the preparation of a medicament, or in methods of diagnostics. Thus, the man skilled in the art, faced with the problem of providing compositions comprising an effective amount of the claimed polypeptide, or a method of diagnosing a *Neisseria meningitidis* infection would not be able to perform it without the need of applying inventive skills and intensive experimentation of undue burden. Therefore, with respect to claims 18-25, the present application does not meet the requirements of Article 5 PCT.

CLAIMS

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence which has at least 99% identity to an amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence is selected from the group consisting of: an amino acid sequence which has at least 99% identity to the amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16.

4. An isolated polypeptide of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

5. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 4, (if necessary when coupled to a carrier) which is capable of raising an immune response which recognises the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide selected from the group consisting of: an amino acid sequence that has at

least 99% identity to an amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has at least 85% identity to an amino acid sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14 or 16 over the entire length of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

7. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: a nucleotide sequence that has at least 97% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO: 2 and a nucleotide sequence which has or at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO: 4, 6, 8, 10, 12, 14 or 16 over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence selected from: the group consisting of a nucleotide sequence which has at least 97% identity to that of SEQ ID NO: 1 and a nucleotide sequence which has at least 85% identity to that of SEQ ID NO: 3, 5, 7, 9, 11, 13 or 15 over the entire length of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15 respectively; or a nucleotide sequence complementary to said isolate polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 selected from the group consisting of: a nucleotide sequence which has at least 97% identity to SEQ ID NO: 1 and a nucleotide sequence which has at least 95% identity to SEQ ID NO: 3, 5, 7, 9, 11, 13 or 15.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide if SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

11. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 obtainable by screening an appropriate library under stringent hybridiziation conditions with a labelled probe having the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15 or a fragment thereof.

13. An expression vector comprising an isolated polynucleotide according to any one of claims 6 to 12.

14. A recombinant live microorganism comprising an expression vector of claim 13.

15. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence that has at least 99% identity to the amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has which has at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 .

16. A process for producing a polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence that has at least 99% identity to an amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has which has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 comprising culturing a host cell of claim 15 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

17. A process for expressing a polynucleotide of any one of claims 6 to 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

18. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

19. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically effective carrier.

20. The vaccine composition according to either one of claims 18 or 19 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.

21. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

22. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 to 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

23. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 to 5 in the preparation of a medicament for use in generating an immune response in an animal.

24. Use of a composition comprising an immunoligically effective amount of a polynucleotide as claimed in any one of claims 6 to 12 in the preparation of a medicament for use in generating an immune response in an animal.

25. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 to 5 and a suitable pharmaceutical carrier.

AMENDED SHEET

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PRIVETT, Kathryn L.
SMITHKLINE BEECHAM
Two New Horizons Court
Brentford
Middlesex TW8 9EP
GRANDE BRETAGNE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	09.04.2001
Applicant's or agent's file reference KP/BM45348	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00135	10/01/2000	15/01/1999

Applicant
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Emslander, S Tel.+49 89 2399-8718

P[redacted]ENT COOPERATION TREA[redacted]

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
KP/BM45348		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00135	10/01/2000	15/01/1999
International Patent Classification (IPC) or national classification and IPC C12N15/31		
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

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   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

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   - I ☒ Basis of the report
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   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01/08/2000	09.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Chavanne, F Telephone No. +49 89 2399 8399

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00135

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-110 as originally filed

Claims, No.:

1-24 as originally filed

Sequence listing part of the description, pages:

1-12, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. ~~1~~-25 partially.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-25 partially.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-5, 13-25
	No:	Claims 6-12
Inventive step (IS)	Yes:	Claims

	No:	Claims	1-25
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The subject-matter of claims 1-25 has been partially searched. Consequently, an opinion with regard to novelty, inventive step and industrial applicability can only be partially formulated, and limited to the invention referred to as invention 1 in the international search report, namely:
Invention 1: polypeptides comprising a sequence homologous to SEQ ID NO:2, immunogenic fragments thereof, polynucleotides encoding said polypeptides, process for their production, vaccines containing them, antibodies specific for said polypeptides, as well as diagnostic and pharmaceutical uses thereof.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: Molecular Microbiology
Vol. 19, No. 5, pp. 1095-1105, 1996
D2: WO 98/02547

3. The scope of claims 6-12 encompasses a nucleic acid molecule comprising the sequence of SEQ ID No. 1. Because of the expression "comprising", said nucleic acid molecule is not necessarily limited to the sequence of SEQ ID No.1, but may also contain other sequences. The genomic DNA of *Neisseria meningitidis*, which has already been isolated (see e.g. D2), is a DNA molecule which comprises the sequence of SEQ ID No.1, and thus, falls within the scope of claims 6-12. Thus, due to the too broad formulation of claims 6-12, the subject-matter of these claims is not novel.
Therefore, claims 6-12 do not meet the requirements of Article 33(2) PCT.

4. The closest prior art to evaluate the inventiveness of the present application is D1. D1 describes a polypeptide from *Neisseria gonorrhoeae* with an amino acid

sequence showing over 97.75% identity with the amino acid sequence of SEQ ID NO:2. The nucleotide sequence of the *comL* gene which encodes said polypeptide is also disclosed, and has been cloned into an expression vector for the specific expression of said polypeptide (abstract; Figures 2-5). Moreover, D1 shows that the homologous counterpart of the *comL* gene is present in *Neisseria meningitidis* and that both genes are highly homologous (abstract; page 1100, column 2, paragraph 3; figure 6).

The subject-matter of claims 1-4 differs from D1 in that the polypeptide of SEQ ID NO. 2 and the isolated polypeptide disclosed in D1 do not have the exact same amino acid sequence. They correspond to homologous products from different *Neisseria* species. However, knowing the *comL* gene from *N. gonorrhoeae*, and being aware of the presence of the *comL* gene highly homologous counterpart in *N. meningitidis*, the man skilled in the art would not require any inventive skill to isolate said gene, and by further applying commonly used technics would automatically come to the subject-matter of claims 1-4 and 6-17. Thus, said subject-matter is not inventive.
The subject-matter of claim 5 further differs from D1 in that D1 does not mention any fragment of the disclosed polypeptide. However, any polypeptide or fragment of a known polypeptide is immunogenic since it triggers an immune response in animals. Thus, the subjcet-matter of claim 5 is not inventive.
The present application does not describe the immunological activity of the claimed polypeptide. However, with the assumption that said polypeptide induces an immunological response, the man skilled in the art, by further applying common knowledge would also come to the subject-matter of claims 18-25. Thus, said subject-matter is not inventive.
Therefore, claims 1-25 do not meet the requirements of Article 33(3) PCT.

VII. Certain defects in the international application

1. The present application does not contain any example showing the best mode for carrying out the invention as required by Rule 5.1(v) (see also Guidelines C-II, 4.9).

2. Although claims 10 and 12 have been drafted as separate independent claims, they both relate to an isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID No.2. The same subject-matter of these claims only differs from each other only in that claim 12 tries to further define said isolated polynucleotide in that it is obtainable by screening with a probe having the sequence of SEQ ID No.1. It appears appropriate to amend said claims by defining the relevant subject-matter in terms of one single independent claim followed by dependent claims covering the optional features (Rule 6.4 PCT).

VIII. Certain observations on the international application

1. The formulation "...comprising..." or "..which comprises..." in claims 6-8 and 10-12 does not clearly define the scope of the claim. Thus, this expression should be replaced with "consisting of" or "which consists of", respectively (Article 6 PCT).

2. Claim 5 refers to an immunogenic fragment capable of raising an immune response which recognises the polypeptide of SEQ ID No. 2. However, the present application does not describe any immunogenic fragment of any polypeptide. Therefore, the subject-matter of claim 5 is not based on the description (Art. 6-support PCT). Since the description does not mention any immunogenic fragment, the man skilled in the art, faced with the problem of providing such a fragment capable of raising an immune response which recognises the polypeptide of SEQ ID No.2, would not be able to provide it without the need of applying intensive experimentations of undue burden. Therefore, with regard to claim 5, the present application does not meet the requirements of Article 5 PCT.
   Moreover, it should be noted that formulations in parentheses are merely optional, and thus, not adapted to limit the scope of the claim.

3. The subject-matter of claims 18-20 refers to a vaccine composition comprising the claimed polypeptide. However, apart from vague declarations on pages 81-92, the specification of the present application neither describes a vaccine composition nor shows that the claimed polypeptide would be effective for a vaccine composition. Moreover, the present application do not provide any evidence that

the claimed polypeptide is adapted for the preparation of immunospecific antibodies, or that the claimed polypeptide can be immunologically effective. Thus, the subject-matter of claims 18-25 is not based on the description (Art. 6-support PCT).

Moreover, with the assumption that the polypeptide described in the present application has the same internal cellular localisation as its homologous counterpart from *N. gonorrhoeae* (see D1, page 1100, column 1, paragraph 2), it is not clear how the immunological characteristics of said polypeptide may be adapted for the preparation of e.g. therapeutical compositions, for its use in the preparation of a medicament, or in methods of diagnostics. Thus, the man skilled in the art, faced with the problem of providing compositions comprising an effective amount of the claimed polypeptide, or a method of diagnosing a *Neisseria meningitidis* infection would not be able to perform it without the need of applying inventive skills and intensive experimentation of undue burden. Therefore, with respect to claims 18-25, the present application does not meet the requirements of Article 5 PCT.

CLAIMS

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence which has at least 99% identity to an amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence is selected from the group consisting of: an amino acid sequence which has at least 99% identity to the amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has at least 95% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16.

4. An isolated polypeptide of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

5. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 4, (if necessary when coupled to a carrier) which is capable of raising an immune response which recognises the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide selected from the group consisting of: an amino acid sequence that has at

least 99% identity to an amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has at least 85% identity to an amino acid sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14 or 16 over the entire length of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

7. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: a nucleotide sequence that has at least 97% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO: 2 and a nucleotide sequence which has or at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO: 4, 6, 8, 10, 12, 14 or 16 over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence selected from: the group consisting of a nucleotide sequence which has at least 97% identity to that of SEQ ID NO: 1 and a nucleotide sequence which has at least 85% identity to that of SEQ ID NO: 3, 5, 7, 9, 11, 13 or 15 over the entire length of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15 respectively; or a nucleotide sequence complementary to said isolate polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 selected from the group consisting of: a nucleotide sequence which has at least 97% identity to SEQ ID NO: 1 and a nucleotide sequence which has at least 95% identity to SEQ ID NO: 3, 5, 7, 9, 11, 13 or 15.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide if SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

11. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15. .

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 obtainable by screening an appropriate library under stringent hybridiziation conditions with a labelled probe having the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15 or a fragment thereof.

13. An expression vector comprising an isolated polynucleotide according to any one of claims 6 to 12.

14. A recombinant live microorganism comprising an expression vector of claim 13.

15. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence that has at least 99% identity to the amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has which has at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 .

16. A process for producing a polypeptide comprising an amino acid sequence selected from the group consisting of: an amino acid sequence that has at least 99% identity to an amino acid sequence of SEQ ID NO: 2 and an amino acid sequence which has which has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 comprising culturing a host cell of claim 15 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

17. A process for expressing a polynucleotide of any one of claims 6 to 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

18. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

19. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically effective carrier.

20. The vaccine composition according to either one of claims 18 or 19 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.

21. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

22. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 to 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

23. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 to 5 in the preparation of a medicament for use in generating an immune response in an animal.

24. Use of a composition comprising an immunoligically effective amount of a polynucleotide as claimed in any one of claims 6 to 12 in the preparation of a medicament for use in generating an immune response in an animal.

25. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 to 5 and a suitable pharmaceutical carrier.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 September 2000 (15.09.00)	
International application No. PCT/EP00/00135	**Applicant's or agent's file reference** KP/BM45348
International filing date (day/month/year) 10 January 2000 (10.01.00)	**Priority date** (day/month/year) 15 January 1999 (15.01.99)
Applicant RUELLE, Jean-Louis et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

01 August 2000 (01.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
KP/BM45348		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/00135	10/01/2000	15/01/1999
Applicant		
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readble form.

   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established by this Authority to read as follows:

   NEISSERIA MENINGITIDIS ANTIGENIC POLYPEPTIDES, CORRESPONDING POLYNUCLEOTIDES AND PROTECTIVE ANTIBODIES

5. With regard to the **abstract,**

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   ☐ as suggested by the applicant.

   ☐ because the applicant failed to suggest a figure.

   ☐ because this figure better characterizes the invention.

   ☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/ 00135

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

Line 1 : after "provides" insert "Neisseria meningitidis"
Line 5 : after "uses" insert "thereof"

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/00135

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24 all partly

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-24 (all partly)

   Polypeptides comprising a sequence homologous to SEQ ID NO:2, immunogenic fragments thereof, polynucleotides encoding said polypeptides, process for their production, vaccines containing them, antibodies specific for said polypeptides, as well as diagnostic and pharmaceutical uses thereof.

2. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:4

3. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:6

4. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:8

5. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:10

6. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:12

7. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:14

8. Claims: 1-24 (all partly)

   As subject 1, but limited to SEQ ID NO:16

INTERNATIONAL SEARCH REPORT

International Application No
PCT [redacted] 00/00135

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/22 A61K39/095 C07K16/12 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DELAHAY RM ET AL: "Involvement of the gonococcal MtrE protein in the resistance of Neisseria gonorrhoeae to toxic hydrophobic agents" MICROBIOLOGY, vol. 143, no. 7, July 1997 (1997-07), pages 2127-2133, XP000907381 figure 1 ---	1-16
X	FUSSENEGGER M ET AL.: "A novel peptidoglycan-linked lipoprotein (ComL) that functions in natural transformation competence of Neisseria gonorrhoeae" MOLECULAR MICROBIOLOGY, vol. 19, no. 5, 1996, pages 1095-1105, XP000907394 figure 2 ---	1-16

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2000	2 [illegible] 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	CUPIDO, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 02547 A (INST NAT SANTE RECH MED ;MAX PLANCK GESELLSCHAFT; SMITHKLINE BEECHAM) 22 January 1998 (1998-01-22) the whole document ---	1-18
A	MARTIN D ET AL: "HIGHLY CONSERVED NEISSERIA MENINGITIDIS SURFACE PROTEIN CONFERS PROTECTION AGAINST EXPERIMENTAL INFECTION" JOURNAL OF EXPERIMENTAL MEDICINE,JP,TOKYO, vol. 185, no. 7, 1997, pages 1173-1183-1183, XP000884332 ISSN: 0022-1007 -----	1-24

INTERNATIONAL SEARCH REPORT

Information [redacted] patent family members

International Application No
PCT [redacted] 00/00135

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9802547 A	22-01-1998	FR 2751000 A	16-01-1998
		AU 3697797 A	09-02-1998
		EP 0951552 A	27-10-1999

Asp Ser Arg Ser Leu His Ser Ser Trp Glu Ala Gly Leu Ile Gly Lys
1730 1735 1740

Asp Asp Glu Trp Tyr Lys Leu Phe Ser Lys Ser Tyr Thr Gln Ala Asp
1745 1750 1755 1760

Leu Ala Leu Gln Ser Tyr His Leu Asn Thr Ala Ala Lys Ser Trp Leu
1765 1770 1775

Gln Ser Gly Asn Thr Lys Pro Leu Ser Glu Trp Met Ser Asp Gln Gly
1780 1785 1790

Tyr Thr Leu Ile Ser Gly Val Asn Pro Arg Phe Ile Pro Ile Pro Arg
1795 1800 1805

Gly Phe Val Lys Gln Asn Thr Pro Ile Thr Asn Val Lys Tyr Pro Glu
1810 1815 1820

Gly Ile Ser Phe Asp Thr Asn Leu Lys Arg His Leu Ala Asn Ala Asp
1825 1830 1835 1840

Gly Phe Ser Gln Glu Gln Gly Ile Lys Gly Ala His Asn Arg Thr Asn
1845 1850 1855

Phe Met Ala Glu Leu Asn Ser Arg Gly Gly Arg Val Lys Ser Glu Thr
1860 1865 1870

Gln Thr Asp Ile Glu Gly Ile Thr Arg Ile Lys Tyr Glu Ile Pro Thr
1875 1880 1885

Leu Asp Arg Thr Gly Lys Pro Asp Gly Gly Phe Lys Glu Ile Ser Ser
1890 1895 1900

Ile Lys Thr Val Tyr Asn Pro Lys Lys Phe Ser Asp Asp Lys Ile Leu
1905 1910 1915 1920

Gln Met Ala Gln Asn Ala Ala Ser Gln Gly Tyr Ser Lys Ala Ser Lys
1925 1930 1935

Ile Ala Gln Asn Glu Arg Thr Lys Ser Ile Ser Glu Arg Lys Asn Val
1940 1945 1950

Ile Gln Phe Ser Glu Thr Phe Asp Gly Ile Lys Phe Arg Ser Tyr Phe
1955 1960 1965

Asp Val Asn Thr Gly Arg Ile Thr Asn Ile His Pro Glu
1970 1975 1980

(2) INFORMATIONS POUR LA SEQ ID NO: 39:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 143 acides aminés
(B) TYPE: acide aminé
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: peptide

(ix) CARACTERISTIQUE:
(A) NOM/CLE: Peptide
(B) EMPLACEMENT: 1..143

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 39:

Met Lys Asn Asn Ile Phe Leu Asn Leu Asn Lys Lys Ser Ile Asn Asn
1 5 10 15

Asn His Phe Val Ile Ser Ile Phe Phe Glu Thr Ile Tyr Gln Phe Glu
20 25 30

Thr Lys Asp Thr Leu Leu Glu Cys Phe Lys Asn Ile Thr Thr Thr Gly
35 40 45

His Phe Gly Val Ile Gly Ala Gln Tyr Glu Lys Ile Asp Ala Thr Arg
50 55 60

Trp Ile Gly Asp Tyr Glu Glu Val Asn Gly Phe Glu Tyr Ile Asp Lys
65 70 75 80

Ala Pro Ser Ile Tyr Phe Ser Val Gly Asp Asp Phe Asn Pro Glu Glu
85 90 95

Leu Ile Ile Pro Ile Asn Leu Ala Tyr His Tyr Phe Asn Ile Ala Ile
100 105 110

Ser Asp Phe Leu Ile Ala His Pro Glu Tyr Gln Lys Lys Cys Lys Glu
115 120 125

Ile Gln Lys Thr Tyr Ser Gln Thr Asn Cys Ser Leu His Glu Thr
130 135 140

(2) INFORMATIONS POUR LA SEQ ID NO: 40:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 833 acides aminés
(B) TYPE: acide aminé
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: peptide

(ix) CARACTERISTIQUE:
(A) NOM/CLE: Peptide
(B) EMPLACEMENT:1..833

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 40:

Val Leu Lys Thr Pro Pro Thr Leu Ala Ala Glu Leu Ser Gly Lys Thr
1 5 10 15

Gly Val Ser Ile Ser Ala Pro Tyr Ala Asn Glu Asn Ser Arg Ile Leu
20 25 30

Leu Ser Thr Thr Asp Ile Ser Ser Glu Asn Gly Lys Ile Lys Ile Gln
35 40 45

Ser Tyr Gly Asp Gln Tyr Tyr Tyr Ala Arg Gln Ser Glu Leu Tyr Thr
50 55 60

Phe Glu Arg Arg Ser Tyr Lys Thr Gly Lys Trp Tyr Asn Arg Lys His
65 70 75 80

Ile Thr Glu Val Lys Glu His Lys Asn Ala Lys Pro Asp Ala Val Asn
85 90 95

Leu Ser Ala Ser Gln Gly Ile Asp Ile Lys Ser Gly Gly Ser Ile Asp
100 105 110

Ala Tyr Ala Thr Ala Phe Asp Ala Pro Lys Gly Ser Ile Asn Ile Glu
115 120 125

Ala Gly Arg Lys Leu Thr Leu Tyr Ala Val Glu Glu Leu Asn Tyr Asp
130 135 140

Lys Leu Asp Ser Gln Lys Arg Arg Arg Phe Leu Gly Ile Ser Tyr Ser
145 150 155 160

Lys Ala His Asp Thr Thr Thr Gln Val Met Lys Thr Ala Leu Pro Ser
165 170 175

Arg Val Val Ala Glu Ser Ala Asn Leu Gln Ser Gly Trp Asp Thr Lys
180 185 190

Leu Gln Gly Thr Gln Phe Glu Thr Thr Leu Gly Gly Ala Thr Ile Arg
195 200 205

Ala Gly Val Gly Glu Gln Ala Arg Ala Asp Ala Lys Ile Ile Leu Glu
210 215 220

Gly Ile Lys Ser Ser Ile His Thr Glu Thr Val Ser Ser Ser Lys Ser
225 230 235 240

Thr Leu Trp Gln Lys Gln Ala Gly Arg Gly Ser Asn Ile Glu Thr Leu
245 250 255

Gln Leu Pro Ser Phe Thr Gly Pro Val Ala Pro Val Leu Ser Ala Pro
260 265 270

Gly Gly Tyr Ile Val Asp Ile Pro Lys Gly Asn Leu Lys Thr Gln Ile
275 280 285

Glu Thr Leu Thr Lys Gln Pro Glu Tyr Ala Tyr Leu Lys Gln Leu Gln
290 295 300

Val Ala Lys Asn Ile Asn Trp Asn Gln Val Gln Leu Ala Tyr Asp Lys
305 310 315 320

Trp Asp Tyr Lys Gln Glu Gly Met Thr Pro Ala Ala Ala Ala Val Val
325 330 335

Val Ile Val Val Thr Val Leu Thr Tyr Gly Ala Leu Ser Ala Pro Ala
340 345 350

Ala Ala Gly Thr Ala Gly Ala Ala Gly Ala Gly Ala Gly Gly Ala Ala
355 360 365

Ala Gly Thr Ala Ala Gly Thr Gly Val Ala Ala Gly Thr Ala Ala Thr
370 375 380

Thr Gly Val Ala Ala Gly Thr Ser Ala Ala Ala Ile Thr Thr Ala Ala
385 390 395 400

Gly Lys Ala Ala Leu Ala Ser Leu Ala Ser Gln Ala Ala Val Ser Leu
405 410 415

Ile Asn Asn Lys Gly Asp Ile Asn His Thr Leu Lys Glu Leu Gly Lys
420 425 430

Ser Ser Thr Val Arg Gln Ala Ala Thr Ala Ala Val Thr Ala Gly Val
435 440 445

Leu Gln Gly Ile Ser Gly Leu Asn Thr Gln Ala Ala Glu Ala Val Ser
450 455 460

Lys His Phe His Ser Pro Ala Ala Gly Lys Leu Thr Ala Asn Leu Ile
465 470 475 480

Asn Ser Thr Ala Ala Ala Ser Val His Thr Ala Ile Asn Gly Gly Ser
485 490 495

Leu Lys Asp Asn Leu Gly Asp Ala Ala Leu Gly Ala Ile Val Ser Thr
500 505 510

Val His Gly Glu Val Ala Ser Lys Ile Lys Phe Asn Leu Ser Glu Asp
515 520 525

Tyr Ile Ala His Lys Ile Ala His Ala Val Ala Gly Cys Ala Ser Ala
530 535 540

Val Ala Asn Lys Gly Lys Cys Arg Asp Gly Ala Ile Gly Ala Ala Val
545 550 555 560

Gly Glu Met Val Gly Glu Thr Leu Leu Asp Gly Arg Asp Val Gly Lys
565 570 575

Leu Ser Pro Gln Glu Arg Gln Lys Val Ile Ala Tyr Ser Gln Ile Ile
580 585 590

Ala Gly Ser Ala Val Ala Leu Val Lys Gly Asp Val Asn Thr Ala Val
595 600 605

Asn Ala Ala Thr Val Ala Val Glu Asn Asn Ser Leu Leu Ala Arg Arg
610 615 620

Arg Val Asn Ile Arg Trp Thr Pro Arg Gln Glu Leu Glu His Glu Tyr
625 630 635 640

Ala Ile Leu Glu Ile Gln Ala Ile Thr Asn Gln Ile Arg Arg Leu Asp
645 650 655

Pro Lys Phe Asn Gly Ile Ala Ile Leu Arg Thr Pro Gly Glu Pro Trp
660 665 670

Thr Arg His Asp Val Gln Thr Tyr Arg Gln Tyr Tyr Asn Gln Leu Arg
675 680 685

Glu Ser Arg Gly Phe Ala Val Glu Pro Ile Tyr Arg Ile Arg Ile Asn
690 695 700

Asn Gly Asn Glu Phe Asn Arg Ile Met Ser Ser Lys Tyr Pro Tyr Asn
705 710 715 720

Glu Leu Tyr Val Ala Asn Pro Lys Ser Ala Thr Gly Tyr Phe Arg Val
725 730 735

Asp Ser Tyr Asp Pro Ala Thr Arg Glu Ile Ile Ser Arg Lys Phe Thr
740 745 750

Gln Phe Ser Gln Ile Gln Glu Ser Thr Gly Ile Gly Tyr Ile Lys Glu
755 760 765

Ala Val Arg Lys Tyr Ser Pro Gly Thr Val Ile Ser Asn Val Pro Ser
770 775 780

Thr Pro Thr Thr Ile Arg Gly Arg Lys Leu Glu Gly Lys Leu Ile Leu
785 790 795 800

Glu Val Pro Ala Gln Val Asn Pro Ile Pro Gln Ser Val Leu Arg Ala
805 810 815

Ala Gln Glu Glu Asn Val Ile Ile Arg Asp Thr Thr Gly Arg Ile Tyr
820 825 830

Lys

(2) INFORMATIONS POUR LA SEQ ID NO: 41:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 833 acides amines
(B) TYPE: acide amine
(D) CONFIGURATION: lineaire

(ii) TYPE DE MOLECULE: proteine
(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 41:

Val Leu Lys Thr Pro Pro Thr Leu Ala Ala Glu Leu Ser Gly Lys Thr
1 5 10 15

Gly Val Ser Ile Ser Ala Pro Tyr Ala Asn Glu Asn Ser Arg Ile Leu
20 25 30

Leu Ser Thr Thr Asp Ile Ser Ser Glu Asn Gly Lys Ile Lys Ile Gln
35 40 45

Ser Tyr Gly Asp Gln Tyr Tyr Tyr Ala Arg Gln Ser Glu Leu Tyr Thr
50 55 60

Phe Glu Arg Arg Ser Tyr Lys Thr Gly Lys Trp Tyr Asn Arg Lys His
65 70 75 80

Ile Thr Glu Val Lys Glu His Lys Asn Ala Lys Pro Asp Ala Val Asn
85 90 95

Leu Ser Ala Ser Gln Gly Ile Asp Ile Lys Ser Gly Gly Ser Ile Asp
100 105 110

Ala Tyr Ala Thr Ala Phe Asp Ala Pro Lys Gly Ser Ile Asn Ile Glu
115 120 125

Ala Gly Arg Lys Leu Thr Leu Tyr Ala Val Glu Glu Leu Asn Tyr Asp
130 135 140

Lys Leu Asp Ser Gln Lys Arg Arg Arg Phe Leu Gly Ile Ser Tyr Ser
145 150 155 160

Lys Ala His Asp Thr Thr Thr Gln Val Met Lys Thr Ala Leu Pro Ser
165 170 175

Arg Val Val Ala Glu Ser Ala Asn Leu Gln Ser Gly Trp Asp Thr Lys
180 185 190

Leu Gln Gly Thr Gln Phe Glu Thr Thr Leu Gly Gly Ala Thr Ile Arg
195 200 205

Ala Gly Val Gly Glu Gln Ala Arg Ala Asp Ala Lys Ile Ile Leu Glu
210 215 220

Gly Ile Lys Ser Ser Ile His Thr Glu Thr Val Ser Ser Ser Lys Ser
225 230 235 240

Thr Leu Trp Gln Lys Gln Ala Gly Arg Gly Ser Asn Ile Glu Thr Leu
245 250 255

Gln Leu Pro Ser Phe Thr Gly Pro Val Ala Pro Val Leu Ser Ala Pro
260 265 270

Gly Gly Tyr Ile Val Asp Ile Pro Lys Gly Asn Leu Lys Thr Gln Ile
275 280 285

Glu Thr Leu Thr Lys Gln Pro Glu Tyr Ala Tyr Leu Lys Gln Leu Gln
290 295 300

Val Ala Lys Asn Ile Asn Trp Asn Gln Val Gln Leu Ala Tyr Asp Lys
305 310 315 320

Trp Asp Tyr Lys Gln Glu Gly Met Thr Pro Ala Ala Ala Ala Val Val
325 330 335

Val Ile Val Val Thr Val Leu Thr Tyr Gly Ala Leu Ser Ala Pro Ala
340 345 350

Ala Ala Gly Thr Ala Gly Ala Ala Gly Ala Gly Ala Gly Gly Ala Ala
355 360 365

Ala Gly Thr Ala Ala Gly Thr Gly Val Ala Ala Gly Thr Ala Ala Thr
370 375 380

Thr Gly Val Ala Ala Gly Thr Ser Ala Ala Ala Ile Thr Thr Ala Ala
385 390 395 400

Gly Lys Ala Ala Leu Ala Ser Leu Ala Ser Gln Ala Ala Val Ser Leu
405 410 415

Ile Asn Asn Lys Gly Asp Ile Asn His Thr Leu Lys Glu Leu Gly Lys
420 425 430

Ser Ser Thr Val Arg Gln Ala Ala Thr Ala Ala Val Thr Ala Gly Val
435 440 445

Leu Gln Gly Ile Ser Gly Leu Asn Thr Gln Ala Ala Glu Ala Val Ser
450 455 460

Lys His Phe His Ser Pro Ala Ala Gly Lys Leu Thr Ala Asn Leu Ile
465 470 475 480

Asn Ser Thr Ala Ala Ala Ser Val His Thr Ala Ile Asn Gly Gly Ser
485 490 495

Leu Lys Asp Asn Leu Gly Asp Ala Ala Leu Gly Ala Ile Val Ser Thr
500 505 510

Val His Gly Glu Val Ala Ser Lys Ile Lys Phe Asn Leu Ser Glu Asp
515 520 525

Tyr Ile Ala His Lys Ile Ala His Ala Val Ala Gly Cys Ala Ser Ala
530 535 540

Val Ala Asn Lys Gly Lys Cys Arg Asp Gly Ala Ile Gly Ala Ala Val
545 550 555 560

Gly Glu Met Val Gly Glu Thr Leu Leu Asp Gly Arg Asp Val Gly Lys
565 570 575

Leu Ser Pro Gln Glu Arg Gln Lys Val Ile Ala Tyr Ser Gln Ile Ile
580 585 590

Ala Gly Ser Ala Val Ala Leu Val Lys Gly Asp Val Asn Thr Ala Val
595 600 605

Asn Ala Ala Thr Val Ala Val Glu Asn Asn Ser Leu Leu Ala Arg Arg
610 615 620

Arg Val Asn Ile Arg Trp Thr Pro Arg Gln Glu Leu Glu His Glu Tyr
625 630 635 640

Ala Ile Leu Glu Ile Gln Ala Ile Thr Asn Gln Ile Arg Arg Leu Asp
645 650 655

Pro Lys Phe Asn Gly Ile Ala Ile Leu Arg Thr Pro Gly Glu Pro Trp
660 665 670

Thr Arg His Asp Val Gln Thr Tyr Arg Gln Tyr Tyr Asn Gln Leu Arg
675 680 685

Glu Ser Arg Gly Phe Ala Val Glu Pro Ile Tyr Arg Ile Arg Ile Asn
690 695 700

Asn Gly Asn Glu Phe Asn Arg Ile Met Ser Ser Lys Tyr Pro Tyr Asn
705 710 715 720

Glu Leu Tyr Val Ala Asn Pro Lys Ser Ala Thr Gly Tyr Phe Arg Val
725 730 735

Asp Ser Tyr Asp Pro Ala Thr Arg Glu Ile Ile Ser Arg Lys Phe Thr
740 745 750

Gln Phe Ser Gln Ile Gln Glu Ser Thr Gly Ile Gly Tyr Ile Lys Glu
755 760 765

Ala Val Arg Lys Tyr Ser Pro Gly Thr Val Ile Ser Asn Val Pro Ser
770 775 780

Thr Pro Thr Thr Ile Arg Gly Arg Lys Leu Glu Gly Lys Leu Ile Leu
785 790 795 800

Glu Val Pro Ala Gln Val Asn Pro Ile Pro Gln Ser Val Leu Arg Ala
805 810 815

Ala Gln Glu Glu Asn Val Ile Ile Arg Asp Thr Thr Gly Arg Ile Tyr
820 825 830

Lys

(2) INFORMATIONS POUR LA SEQ ID NO: 42:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 162 acides aminés
(B) TYPE: acide aminé
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: peptide

(ix) CARACTERISTIQUE:
(A) NOM/CLE: Peptide
(B) EMPLACEMENT:1..162

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 42:

Met Lys Lys Asp Ile Phe Tyr Cys Glu Gln Trp Ser Tyr Gly Tyr Lys
1 5 10 15

Arg Leu His Lys Pro Phe Ser Glu Lys Gln Ala Glu Glu Lys His Leu
20 25 30

Lys Gly Glu Leu Tyr Thr Ala Val Ile Gly Ser Ala Thr Gln Pro Glu
35 40 45

Tyr Val Ile Thr Leu Arg Glu Glu Val Gly Phe Phe Ser Val Asn Phe
50 55 60

Phe Asp Lys Phe Gly Arg Asp Tyr Leu Thr His Gln Phe Gln Lys Tyr
65 70 75 80

Ser Asn Ser Asn Tyr Tyr Phe Leu Ser Met Ala Val Trp Arg Asp Tyr
85 90 95

Ile Thr Leu Glu Ser His Asp Leu Ala Glu Gly Tyr Thr Tyr Phe Phe
100 105 110

Asn Glu Asn Thr Asp Asp Cys Tyr Val Leu Lys Gln Asp Phe Ile Asn
115 120 125

Asn Glu Arg Tyr Glu Lys Thr Glu Leu Tyr Ser Gln Lys Asp Lys Val
130 135 140

Ile Leu Phe Pro Lys Phe Gly Glu Tyr Asp Leu Val Leu Asn Pro Asp
145 150 155 160

Ile Ile

(2) INFORMATIONS POUR LA SEQ ID NO: 43:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 90 acides aminés
(B) TYPE: acide aminé
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: peptide

(ix) CARACTERISTIQUE:
(A) NOM/CLE: Peptide
(B) EMPLACEMENT:1..90

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 43:

Met Asn Lys Arg Met Lys Met Cys Pro Ala Cys Gln Gln Gly Tyr Leu
1 5 10 15

Tyr His Ser Lys Pro Lys Tyr Leu His Asp Glu Ile Ile Leu Cys Asp
20 25 30

Glu Cys Asp Ala Val Trp Leu Lys Gly Met Asn Ile Phe Tyr Gly Glu
35 40 45

Tyr Glu Lys Asp Phe Tyr Ser Tyr Val Pro Phe Met Glu Ser Gln Gly
50 55 60

Ile Thr Ser Glu Cys Ile Trp Glu Gly Asp Leu Phe Asp His Pro Tyr
65 70 75 80

Tyr Glu Asp Glu Asn Ser Asn Asp Met Asp
85 90

(2) INFORMATIONS POUR LA SEQ ID NO: 44:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 313 acides aminés
(B) TYPE: acide aminé
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: peptide

(ix) CARACTERISTIQUE:
(A) NOM/CLE: Peptide
(B) EMPLACEMENT: 1..313

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 44:

Met Ser Ala Thr Glu Ile Glu Lys Ala Lys Ala Lys Ile Thr Ala Tyr
1 5 10 15

Ser Lys Leu Val Ala Gly Thr Ala Ser Ala Val Val Gly Gly Asp Val
20 25 30

Asn Thr Ala Ala Asn Ala Ala Gln Ile Ala Val Glu Asn Asn Thr Leu
35 40 45

Tyr Pro Arg Cys Val Gly Ala Lys Cys Asp Glu Phe Gln Lys Glu Gln
50 55 60

Gln Lys Trp Ile Arg Glu Asn Pro Glu Glu Tyr Arg Glu Val Leu Leu
65 70 75 80

Phe Gln Thr Gly Phe Ile Pro Ile Ile Gly Asp Ile Gln Ser Phe Val
85 90 95

Gln Ala Gln Thr Ala Ala Asp His Leu Phe Ala Leu Leu Gly Val Val
100 105 110

Pro Gly Ile Gly Glu Ser Ile Gln Ala Tyr Lys Val Ala Lys Ala Ala
115 120 125

Lys Asn Leu Gln Gly Met Lys Lys Ala Leu Asp Lys Ala Ala Thr Val
130 135 140

Ala Thr Ala Gln Gly Tyr Val Ser Lys Thr Lys Ile Lys Ile Gly Gln
145 150 155 160

Thr Glu Leu Arg Val Thr Ala Ala Thr Asp Lys Gln Leu Leu Lys Ala
165 170 175

Ile Gly Glu Gly Arg Asp Thr Thr Gly Lys Met Thr Glu Gln Leu Phe
180 185 190

Asp Ser Leu Ala Lys Gln Asn Gly Phe Arg Val Leu Ser Gly Gly Lys
195 200 205

Tyr Gly Gly Asn Asn Gly Phe Asp His Val Trp Gln Ala Ala Asp Gly
210 215 220

Ser Val Val Leu Ile Val Glu Ser Lys Gln Ile Arg Asn Gly Thr Val
225 230 235 240

Gln Leu Asn Pro Asn Gly Ala Gly Gly Tyr Thr Gln Met Ser Glu Asp
245 250 255

Trp Ile Arg Gln Val Leu Asp Gln Leu Pro Asp Gly Ser Pro Ala Lys
260 265 270

Ala Ala Val Phe Lys Ala Asn Lys Asn Gly Thr Leu Lys Thr Ala Ile
275 280 285

Ala Gly Val Asp Arg Gln Thr Gly Lys Ala Val Ile Leu Pro Val Lys
290 295 300

Val Pro Ser Lys Thr Asn Ile Arg Arg
305 310

(2) INFORMATIONS POUR LA SEQ ID NO: 45:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 311 acides aminés
(B) TYPE: acide aminé
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: peptide

(ix) CARACTERISTIQUE:
(A) NOM/CLE: Peptide
(B) EMPLACEMENT:1..311

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 45:

Met Gly His Asn Met Met Thr Thr Gln Lys Trp Tyr Glu His Ile Thr
1 5 10 15

Asn Val Ile Ile Gly Asn Thr Ala Asn Phe Asn Ser Gly Cys Leu Asp
20 25 30

Ser Ile Asp Tyr Val Asp Glu Arg Lys Gly Val Pro Leu Ala Ala Met
35 40 45

Gln His Ile Phe Met Asp Val Arg Ala Ala Ala Ser His Ala Tyr Leu
50 55 60

Phe Glu His Asp Leu Lys Lys Phe Lys Gln Tyr Ala Tyr Val Ala Gly
65 70 75 80

Lys Leu Gly Val Leu Leu Ser Val Asn Ser Thr Asp Pro Glu Pro Phe
85 90 95

Phe Phe Pro Cys Asp Met Leu Asn Ile Gln Asn Pro Met Phe Leu Met
100 105 110

Leu Met Ser Asp Ser Pro Gln Leu Arg Glu Phe Leu Val Arg Asn Ile
115 120 125

Asp Asn Ile Ala Asn Asp Thr Glu Ala Phe Ile Asn Arg Tyr Asp Leu
130 135 140

Asn Arg His Met Ile Tyr Asn Thr Leu Leu Met Val Glu Gly Lys Gln
145 150 155 160

Leu Asp Arg Leu Lys Gln Arg Ser Glu Lys Val Leu Ala His Pro Thr
165 170 175

Pro Ser Lys Trp Leu Gln Lys Arg Leu Tyr Asp Tyr Arg Phe Phe Leu
180 185 190

Ala Phe Ala Glu Gln Asp Ala Glu Ala Met Lys Ala Ala Leu Glu Pro
195 200 205

Leu Phe Asp Lys Lys Thr Ala Arg Met Ala Ala Lys Glu Thr Leu Ser
210 215 220

Tyr Phe Asp Phe Tyr Leu Gln Pro Gln Ile Val Thr Tyr Ala Lys Ile
225 230 235 240

Ala Ser Met His Gly Phe Asp Leu Gly Ile Asp Gln Glu Ile Ser Pro
245 250 255

Arg Asp Leu Ile Val Tyr Asp Pro Leu Pro Ala Asp Glu Tyr Gln Asp
260 265 270

Ile Phe Asp Phe Met Lys Gln Tyr Asp Leu Ser Tyr Pro Tyr Glu Tyr
275 280 285

Leu Gln Asp Trp Ile Asp Tyr Tyr Thr Phe Lys Thr Asp Lys Leu Val
290 295 300

Phe Gly Asn Ala Lys Arg Glu
305 310

(2) INFORMATIONS POUR LA SEQ ID NO: 46:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 21 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 46:

GCCACCGGTA CGGAAACTGA A 21

(2) INFORMATIONS POUR LA SEQ ID NO: 47:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 30 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 47:

CCTGAATTCA TGTCTATTCC ATTTTGAAGA 30

(2) INFORMATIONS POUR LA SEQ ID NO: 48:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 31 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 48:

CCGAGATCTT TAACCCTTTG GGCTTAAGCG A 31

(2) INFORMATIONS POUR LA SEQ ID NO: 49:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 29 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 49:

GGGAGATCTC CCGCTCGTGT TGTGCATTA 29

(2) INFORMATIONS POUR LA SEQ ID NO: 50:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 28 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 50:

AAGAGATCTG CAGCCAAGGC TCTCGAAA 28

(2) INFORMATIONS POUR LA SEQ ID NO: 51:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 26 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 51:

GGGAGATCTC AGGCTGCCGC CGTTGA 26

(2) INFORMATIONS POUR LA SEQ ID NO: 52:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 28 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 52:

GGGAGATCTC ACCCCAAGAA CGCCAAAA 28

(2) INFORMATIONS POUR LA SEQ ID NO: 53:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 31 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 53:

GGGAGATCTG AACGTATAGT AATCTATCCA A 31

(2) INFORMATIONS POUR LA SEQ ID NO: 54:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 12 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 54:

AGTGGCTCCT AG 12

(2) INFORMATIONS POUR LA SEQ ID NO: 55:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 24 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 55:

AGCACTCTCC AGCCTCTCAC CGAG 24

(2) INFORMATIONS POUR LA SEQ ID NO: 56:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 12 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 56:

AGTGGCTCTT AA 12

(2) INFORMATIONS POUR LA SEQ ID NO: 57:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 10 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 57:

AGTGGCTGGC 10

(2) INFORMATIONS POUR LA SEQ ID NO: 58:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 24 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO:58:

AGCACTCTCC AGCCTCTCAC CGAC 24

(2) INFORMATIONS POUR LA SEQ ID NO: 59:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 12 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (genomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 59:

GTACTTGCCT AG 12

(2) INFORMATIONS POUR LA SEQ ID NO: 60:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 24 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 60:

ACCGACGTCG ACTATCCATG AACG 24

(2) INFORMATIONS POUR LA SEQ ID NO: 61:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 12 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 61:

GTACTTGCTT AA 12

(2) INFORMATIONS POUR LA SEQ ID NO: 62:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 10 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 62:

GTACTTGGGC 10

(2) INFORMATIONS POUR LA SEQ ID NO: 63:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 24 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 63:

ACCGACGTCG ACTATCCATG AACC 24

(2) INFORMATIONS POUR LA SEQ ID NO: 64

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 12 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 64:

AATTCTCCCT CG

(2) INFORMATIONS POUR LA SEQ ID NO: 65

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 24 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 65:

AGGCAACTGT GCTATCCGAG GGAG

(2) INFORMATIONS POUR LA SEQ ID NO: 66:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 140 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 66:

GATCAACTTT TCCCTGTTTG TCCCATTACC GGTTTGAATG AACCGATTGC GCGCCGCGCG 60

TGTTGTTGGA CATTACCTGC GATTCAGACG GTACGATTGA CCACTACATC GAGGAGAACG 120

GCAATCAGGG TACAATGCTA 140

(2) INFORMATIONS POUR LA SEQ ID NO: 67:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 192 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 67:

GATCCGCGTA CTTGGTTTTT CATATTTTGC ATAGTCTTGT CGGTCGGGCA TCTTCCCCGA 60

CATCATCTAA ATTTGTCTTT ATTGGTTTTT ACGCCACTCA TTGCGGATAA ACAATATTCC 120

GCCTTGCCGT CGCGAATGTT CAAGCTAGCC TGCATCACCG TAATCAGGTT GCCCGTTACC 180

GAGCCTTCGA GA 192

(2) INFORMATIONS POUR LA SEQ ID NO: 68:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 188 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 68:

GATCCGGCTG CCCGACGCGC GCAAAATTGC CGCCGAGGAA AGCGCGCACA ACCACGACGG 60

CAAAACCAGC GTATGGCAAT ACAAACATCT CGTGTTCGGT ACGGCAGGCA TTTTCTGCTA 120

TGTCGGCGCG GAGGTGTCTA TCGGTTCGTT GATGGTCAAC GTATTGGGTT ATCTGAAAGG 180

GCTGGATC 188

(2) INFORMATIONS POUR LA SEQ ID NO: 69:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 304 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 69:

GATCCCCCAC TTTACCTCGG GCAGATTTTG CGCGTTCATT ACAATAGCGT ATTTATGCGT 60

TTGCGTTTGC GCTTGCCGCT GCCCCCCCCC CGCCGGTATG GGAAAACATC AATATGGCGG 120

TATAAAGCGC GGTATGGCGG AAAACCTGCC GTTTCCAAGT TTTATTCATC TTTTATTCCT 180

TGAGTTTGCC TTCACGGGAC GGGGCGGCGC GCGGAACGCG GGGTTCGGTA AACCGCCCGA 240

TTCCGCGCCC GCCGAATTGC TGATTGAAAA GCTTACTTCC CCATTTTAAC TTTGCACACT 300

GATC 304

(2) INFORMATIONS POUR LA SEQ ID NO: 70:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 243 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 70:

GATCAGACCC ATTTTCAGCG CACCGTAAGC GCGGATTTTC TCGAATTTTT CCAAAGCTGC 60

GGCATCGTTG TTGATGTCGT CTTGCAACTC TTTGCCCGTG TAGCCCAAGT CGGCGGCATT 120

CAGGAAAACG GTCGGAATGC CCGCGTTGAT GAGCGTGGCT TTCAAACGGC CTATATTCGG 180

CACATCAATT TCATCGACCA AATTGCCGGT TGGGAACATA CTGCCTTCGC CGTCGGCTGG 240

ATC 243

(2) INFORMATIONS POUR LA SEQ ID NO: 71

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR. 236 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 71:

CGGCGGCGTAGTccgccGcgACAGCGTTACCATAAGCGGGACAGACTACACCCCTTTATCT
AACCCGCAAAGTTTGGATACGGAATTAAAATGGTTGCTTCAAGAAGCTCCCGAAATAG
AAAATCCTTTCGACCGCGCCGTTTATCTCCATAATAATTTGGCGTATCTTCAATATTTT
AAAGATTGCAATAAACGTACTGCCAGAAACTGCATGACCTTGTCGCTGATGCGCTCCG

(2) INFORMATIONS POUR LA SEQ ID NO: 72:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 280 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 72:

CGGTCAATCA CAAGAAAGTC AGCCGTCTGA TGGCGAAGAC GGGGCTGAAG GCAGTGATAT 60

GGCGGCGCAA ATACCGCTCG TTCAAAGGAG AAGTCGGCAA AATTGCGCCG AATATCCTGC 120

GACGCTGTTT CCATGCAGAA AAGCCGAATG AGAAATGGGT AACGGACGTT GCCGAGTTCA 180

ATGTAGGCGG AGAAAAGATA TACCTTTCTC CGATTATGGA TTTGTTTAAC GGGGAAATCG 240

TCAGTTACCG TATTCAGACC CGCCCGACTT TCGATTTGGC 280

(2) INFORMATIONS POUR LA SEQ ID NO: 73:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 120 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 73:

CGGTCAGAAA CAGGCAAGGT AATGAAAATG CCTGAGGCAC GGACTGTGCT GCGAACGAAA 60

ACTCCTTACC GAAGTCTTCT ATACCCAGGC TCAATAGCCG CTCAAGGAGA GAGCTATCAT 120

(2) INFORMATIONS POUR LA SEQ ID NO: 74:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 120 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 74:

CGGTCAGAAA CAGGCAAGGT AATGAAAATG CCTGAGGCAC GGACTGTGCT GCGAACGAAA 60

ACTCCTTACC GAAGTCTTCT ATACCCAGGC TCAATAGCCG CTCAAGGAGA GAGCTATCAT 120

(2) INFORMATIONS POUR LA SEQ ID NO: 75:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 152 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: lineaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 75:

CGGTGTTTTT CTTAACAATT CGCCGACTTC ATGGCGATAT TTAAGTGACA GTTGCTCCGC 60

CCACGCACTT GCGCCGAACT CAGCACCACG ACATTATACT GATTATGCAC ATCGGCAAGA 120

TCAAACTGAC CTATCGTAGT ATCGCAGACT GT 152

(2) INFORMATIONS POUR LA SEQ ID NO: 76

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 381 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 76:

CGGGAGGTTTTGTGCATCCTGATACCGATCGGTTGTTGTTGCTCAAAGGACAGAAGGC
CGCTGATAAACGAGATTACCTGTTTGTCGCTATTGACGATTTTTATACTCTGCCATTTT
GCCAGACAAAACCGCAGACAGTGCTGCCAAGTTTCTGACCGAACATCTGGCCGACCCC
TGCTTGTACCTGATTGAGTACGCTTACTCTGACAATGATAGGTAATATAAAGAGCCGTC
CAACATGCTTTCGGTGCAGTTTGTTATGATAATGGGATTGGTTGGAGGCTTGCCCGATT
TGCTTGTCCGCAGACCAACGGTAAGGCGGAGCGGGTTATCCGTACCTTGATGGAGATG
TGGCATGAGGAACAGTCGTTTGACAGACCG

(2) INFORMATIONS POUR LA SEQ ID NO: 77

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 269 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 77:

CGGAGCATAA AATCGTTATT AAAGATAATG GTATAGGAAC GAGCTTCGAT GAAATCAATG 60

ATTTTTATTT GAGAATCGGT CGGAACAGAA GGGAAGAAAA ACAAGCCTCC CCGTGCGGAA 120

GAATTCCAAC GGGTAAAAAA GGCCTTGGTA AATTGGCATT ATTCGGGCTT GGCAACAAAA 180

TTGAAATTTC TACTATCCAG GGAAACGAAA GGGTTACTTT TACTTTGGAT TATGCAGAGA 240

TTCGAAGAAG CAAGGGTATT TATCAACCG 269

(2) INFORMATIONS POUR LA SEQ ID NO: 78

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 203 paires de bases
(B) TYPE: nucléotide

(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 78:

CGGATGAAAACGGCATACGCgcCAAAGTATTTACGAACATCAaAGGCTTGAAGATACCG
CACACCTACATAGAAACGGACGCGAAAAAGCTGCCGAAATCGACAGATGAGCAGCTTT
CGGCGCATGATATGTACGAATGGATAAAGAAGCCCGAAAATATCGGGTCTATTGTCAT
TGTAGATGAAGCTCAAGACGTATGGCCG

(2) INFORMATIONS POUR LA SEQ ID NO: 79:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 229 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 79

CGGTTTCAGG TTGTCGCGAA GGCTCGGTAA CGGGCAACCT GATTACGGGT GATGCAGGCA 60

GCTTGAACAT TCGCGACGGC AAGGCGGAAT ATGTTTATCC GCAATGAGTG GCGTAAAAAC 120

CAATAAAGAC AAATTTAGAT GATGTCGGGG AAGATGCCCG ACCGACAAGA CTATGCAAAA 180

TATGAAAAAC CAAGTACGCG GATCAGGCAT GGATGCACGA TCCAATCCG 229

(2) INFORMATIONS POUR LA SEQ ID NO: 80:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 207 paires de bases
(B) TYPE: nucléotide

(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: lineaire

(ii) TYPE DE MOLECULE: ADN (genomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 80.

CGGGTCGCTT TATTTTGTGC AGGCATTATT TTTCATTTTT GGCTTGACAG TTTGGAAATA 60

TTGTGTATCG GGGGGGGGTA TTTGCTGACG TAAAAAACTA TAAACGCCGC GCAAAATATG 120

GCTGACTATA TTATTGACTT TGATTTTGTC CTGCGCGGTG ATGGATAAAA TCGCCAGCGA 180

TAAAGAATTT GCGAGAACCT GATGCCG 207

(2) INFORMATIONS POUR LA SEQ ID NO: 81 :

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 224 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 81.

CGGCAACGAT TTGAGCTATC GCGGTTACGA CATTCTGGAT TTGGCACAAA AATGCGAGTT 60

TGAAGAAGTC GCCCACCTGC TGATTCACGG CCATCTGCCC AACAAATTCG AGCTGGCCGC 120

TTATAAAACC AAGCTCAAAT CCATGCGCGG CCTGCCTATC CGTGTGATTA AAGTTTTGGA 180

AAGCCTGCCT GCACATACCC ATCCGATGGA CGTAATGCGT ACCG 224

(2) INFORMATIONS POUR LA SEQ ID NO: 82:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 212 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (genomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 82.

CGGGAACAGC CATTGCCCAC GCCCACGCCC CCCAAGAAAG ACGGAAACTA CTGCCTAAAT 60

TTTCGGCAAT CAAGTTGACG ATTAAAGGGT TGGGGGCAGT TGCAGTAATA AACATAGCCG 120

ACGAAATGGG ATTGGAATGA TAGTTGACCA AAGCCAAATA TTTACCCATC TTGCCTTCTG 180

TGCCTTTTGC GGGATTGGAG CCGTAACTGC CG 212

(2) INFORMATIONS POUR LA SEQ ID NO: 83

(i) CARACTERISTIQUES DE LA SEQUENCE.
(A) LONGUEUR: 353 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (genomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 83

CGGGAATTCT GAGCAGAATG AAAGAAAGCA GGCTTGATAA TTTCATAAAG TTATTGGAAG 60

AAAAAGGATT TACCGTCCAT TTCGGTATTC ACAATACGGC TGATTACGGA ATTCCCCAAA 120

GCCGTAAAAG ATTTACGTTA ATTGCAAACA GAATAACCAA AGAAAAGCTG GAACCAGTCA 180

AGTATTCGGG CAAACGGCTT ACGGTAGCCG ATGTTTTGGG AATGGAAATG GCTTTCCCAA 240

CATTATTGCA GGACACCAAG ACGAAACGGA TTTTATGCAT AGCTGTGCGG GAATTATCTG 300

ATATCACTTG AACGATTGGC TTGATACCTA AAAACGGAGG AACCGTTGGC TTT 353

(2) INFORMATIONS POUR LA SEQ ID NO: 84:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 308 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 84:

AATTCCGTAT CCAAACTTTG CGGGTTAGAT AAAGGGGTGT AGTCTGTCCC GCTTATGGTA 60

ACGCTGTCGC GGCGGACTAC GCCCGGAGCC TTTTTCCAGT AAGTTTTCGG AAATCAGGCT 120

GTGGGTGGTT TTTAAGAAAT CCAACCAGTC AAACGGCTCG GGGCTGTCCA AACCGGACAC 180

AGGTGCCGGT AACTTTCCCT CAGGTTGATT AACATTACGG CATCCGAATA TAACTTCCCG 240

CCTGCGGTTT GCCCGAGTTT AAGCAATGCC TGCGTATCGT ATTGATTATA AAGTGTTTCC 300

TTCCAATT 308

(2) INFORMATIONS POUR LA SEQ ID NO: 85:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 104 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 85:

AATTCGTGTG CCGCGTCGAC AAACCGCTGA CGTAGCGGAT GTCTCATGCC ACGTTTCAAA 60

GCAGGTTGAT GGCGGTTAGC AACCCTCTGA TTTCACTGGG ATAT 104

(2) INFORMATIONS POUR LA SEQ ID NO: 86:

(i) CARACTERISTIQUES DE LA SEQUENCE.
(A) LONGUEUR: 89 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 86:

AATTGCGTAG AGTGGGCTTC AGCCACGTTT TTTCTTTTTC GGTCGTTGAT TGGTGGGCTG 60

AACCACTTGT TTCGGAAATC CGTATCATG 89

(2) INFORMATIONS POUR LA SEQ ID NO: 87:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 273 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 87:

AATTTCCACC TATGCCCTAC GCAGCGATTA TCCGTGGTTT ACCCAAAGGG TGATTATGGC 60

AAAAGCGCGG GGTTGAGCGA CCGCCTTTTG TTGCCGGCGT TCAAACGGGT TTTGATAGGA 120

AATGCAGGCA CGAAGCCTCG GCTGATTGTG ATGCACCTGA TGGGTTCGCA CAGTGATTTT 180

TGCACACGTT TGGATAAGGA TGCGCGGCGG TTTCAGTATC AAACTGAAAA AATATCCTGC 240

TATGTTTCCA TCAATCGCGC AAACCGATAA ATT 273

(2) INFORMATIONS POUR LA SEQ ID NO: 88:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 270 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 88:

AATTCTTCCG CACGGGGAGG CTTGTTTTTC TTCCCTTCTG TTCCGACCGA TTCTCAAATA 60

AAAATCATTG ATTTCATCGA AGTTCATTCC TATACCATTA TCTTTAATAA CGATTTTATG 120

CTCCGGTTTA TCGAATAACC TAACTTCCAC TTCCGTAGCA CATGCATCGT AGGCATTCGC 180

TATCAACTCG GCAATCGCAG GAACAGTGTG CGAATACAAT CTTTACACCC AAATGTTCGA 240

TTACGGTTGG CTCGAAACTC AATTTCAATT 270

(2) INFORMATIONS POUR LA SEQ ID NO: 89:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 267 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 89:

AATTATGAAC ACACGCATCA TCGTTTCGGC TGCGTTCGTT GCGTTGGCAT TAGCAGGTTG 60

CGGCTCAATC AATAATGTAA CCGTTTCCGA CCAGAAACTT CAGGAACGTG CCGCGTTTGC 120

CTTGGGCGTC ACCAATGCCG TAAAAATCAG CAACCGCAGC AATGAAGGCA TACGCATCAA 180

CTTTACCGCA ACTGTGGGTA AGCGCGTGAC CAATGCTATG TTACCAGTGT AATCAGCACA 240

ATCGGCGTTA CCACTTCCGA TGCAATT 267

(2) INFORMATIONS POUR LA SEQ ID NO: 90:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 234 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 90:

AATTTTTATT TGGTTCGTAG TCATTTTGTG CAACTGAACG ATATTCGTTT TCATCATTGC 60

TAACGTCTAG TGCCCATTGT GGCCCGTAAT AAGAGATTTC GTCTCCTTTT ACATGTTTGA 120

CGCTGACGGC ATACTGGGGA TCGATGACGG ATAATGTACG TCTGTTGACA TCTGCAACGC 180

TAAATCAATC ATCGGTATTG GATAATGCGT TGCCGATGTT TTGACTTGTA TGTT 234

(2) INFORMATIONS POUR LA SEQ ID NO: 91:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 295 paires de bases
(B) TYPE: nucléotide

(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: lineaire

(ii) TYPE DE MOLECULE: ADN (genomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 91:

AATTCGGCCG GCTGTGTCAA ATAATGCGTT ACTTTGGCCG GGTCTTGTTC TTTGTAAGTG 60

GTGGTCTTTT TTTGCGCGTT ATCCCCATCT GTTTGAGTGC ATAGCAAATG GTGGCTGCCG 120

TACAATCAAA TGTTTGGCGT TCATGCAGAT AGGCATCATG GTGTTGCCCA ATATATTGAG 180

CCGGTTTTTG CCTATCCGAT TTGACGGCAT TTAGACCGGT AACTTGATGT TTTAAGCTGC 240

CTGTTTGTTT AAAGGCGAAT CCACAAGTAA AGCGTGTTTC TTGACAGGTT AAACG 295

(2) INFORMATIONS POUR LA SEQ ID NO: 92:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 259 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 92:

AATTGTGTAT ATCAAGTAGG ATGGGCATTT ATGCCTGACC TACAAAACCA AAAACAACCT 60

ACCACCCTTA ATCAACTCCA CAAACCCTCT TCAGACAACC TCGTTTTTTG AAAAACAATC 120

TGTAAACAGA TAACTGCTGA AGAATACCGT TGCCGAGCCC CAAAACCCGT ACTGCAACTT 180

TTATTGTGAA CTTCCCATTA TGAGAAAATC CCTTTTCGTC CTCTTTCTGT ATTCGTCCCT 240

ACTTACTGCC AGCGAAATT 259

(2) INFORMATIONS POUR LA SEQ ID NO: 93:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 379 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 93:

AATTGCACCA CGCGATGATG GGTACGCCTC TGTTGCCATT GCGACCGCCG CCGCCGTGCC 60

CGGTACGCTG GTCAACCTTG CCGCGGCGGA ACGGGTAAAG AAGTGCGCTT CGGGCATCCT 120

TCCGGTACAT TGCGCGTCGG TGCAGCGCCG AATGTCAGGA CGGACAATGG ACGGCCACCA 180

AAGCGGTTAT GAGCCGCAGC GCACGCGTGA TGATGGAAGG TTGGGTCAGG GTGCCGGAAG 240

ATTGTTTTTA AATTGGACGG CGAACCGGTC TATTCGTATT GGCGTTATAC CGCCGCAAAG 300

GCAGACCTTG AAACTGGTGC GTGCCGTGCA GGGCATGTAC GGCTATGTGT GCGTGGCGGG 360

CGGATTTGAT GTGCGGAAT 379

(2) INFORMATIONS POUR LA SEQ ID NO: 94:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 308 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 94:

AATTTGTTGG GCAGATGGCC GTGAATCAGC AGGTGGGCGA CTTCTTCAAA CTCGCATTTT 60

TGTGCCAAAT CCAGAATGTC GTAACCGCGA TACGTCAAAT CGTTGCCGGT ACGCAACGGT 120

ACACAAAGCG GTATTACCGG CCGCAACGCC AGAAAGCGCA ACGGATTTTT AGGTTTGAGG 180

GTCGGGGTTT GAGTAGTTTC AGTCATGGTA TTTCTCCTTT GTGTTTTTAT GGGTTTCGGG 240

TTTTCAGACG ACCGATGCGG ATTTGTTGAA AGGCAGTCTG AAAGCGGTAA ATCATTTTTG 300

AAACAATT 308

(2) INFORMATIONS POUR LA SEQ ID NO: 95:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 286 paires de bases
(B) TYPE. nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 95:

AATTCGGAGG AGCAGTACCG CCAAGCGTTG CTCGCCTATT CCGGCGGTGA TAAAACAGAC 60

GAGGGTATCC GCCTGATGCA ACAGAGCGAT TACGGCAACT TGTCCTACCA CATCCGTAAT 120

AAAAACATGC TTTTCATTTT TTCGGCAAGC AATGACGCAC AAGCTCAGCC CAACACAACT 180

GACCCTATTG CCATTTTATG AAAAAGACGC TCAAAAAGGC ATTATCACAG TTGCAGGCGT 240

AGACCGCAGT GGAGAAAAGT TCAATGGCTC CAACCATTGC GGAATT 286

(2) INFORMATIONS POUR LA SEQ ID NO: 96:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 238 paires de bases

(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 96:

AATTTGGATA CGTTGGAAAA GGGATATTTG ATTGGGAATG GGATGAAGAT AAGCGTAGAT 60

GAGTTGGGGA AAAAAGTGTT AGAACATATC GGTAAGAATG AACCGTTATT GTTGAAAAAT 120

CTACTGGTTA ACTTCAATCA GGGAAAACAT GAAGAAGTTA GGAAGTTGAT TTATCAGTTG 180

ATAGAGTTAG ATTTTCTGGA ACTTTTGTGA GGGATTCTAT GAAAAACTGG AAGCAATT 238

(2) INFORMATIONS POUR LA SEQ ID NO: 97:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 322 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 97:

AATTCGGCAC GCAGGTTTTC TAAAAAAAGG CCGTTGATGA CTTTGTCGAT ATTGGCGGCT 60

TCGGTGTAGT GCGCGCCCGC TTCGGCCGCT CTTGCGCGTC CATGACGGAT TGGAAGAGCG 120

TGCCGAAGAT TTCTGGACTG ATGTTGCGCC AGTCGAAATT GCCGACACGG GAGGAATACC 180

TGCCAACAAG AGTGCAGGCA GCGTAATCAA ACCACCCCCA CCCGCAATCG CATCGATAAA 240

TCCGGCAATC ATCGCAACCA AACCCAAAGC GAGTATTATG TATAAATCTT CCATGTTTCT 300

TAATCCTGTT AACTTGCACC AA 322

(2) INFORMATIONS POUR LA SEQ ID NO: 98:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 316 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 98

AATTTGTCGG CAATCTTCCC GGGTCGCTTT ATTTTGTGCA GGCATTATTT TTCATTTTTG 60

GCTTGACAGT TTGGAGATAT TGTGTATCGG GGGGGGGTAT TTGCTGACGT AAAAAACTAT 120

AAACGCCGCA GCAAAATATG GCTGACTATA TTATTGACTT TGATTTTGTC CTGCGCGGTG 180

ATGGATAAAA TCGCCAGCGA TAAAGATTTG CGAGAACCTG ATGCCGGCCT GTTGTTGAAT 240

ATTTTCGACC TGTAATTACG ATTTGGCTTC CGCGCCGGCA CAATATGCCG CCAAGCGGCG 300

CCCACATTTT GGAAGC 316

(2) INFORMATIONS POUR LA SEQ ID NO: 99:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 217 paires de bases
(B) TYPE: nucléotide
(C) NOMBRE DE BRINS: simple
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(iii) HYPOTHETIQUE: NON

(iv) ANTI-SENS: NON

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 99:

AATTCGGACA GTATGAATAC AGCGGATTAA TACAAGGTAA GTTCATTACA ACGGAAAAAC 60

CTTTAAAGAA TAATATGAAA GGTATTACCT TGTTTGCCAA CGGGAATGGT AAATATGCCC 120

GAGTTTTTCA CTGAATAGCG AATCCAGCCA TTTCTATTCA TATTTGACTG GATGGCTGAA 180

TGTGGACTTT ATAGATAATG ACGATGAAGA TTTAATT 217

REVENDICATIONS

1/ ADN caractérisés en ce qu'il s'agit de tout ou partie de gènes, avec leur phase de lecture, présents chez *Neisseria meningitidis*(désignée ci-après par Nm), mais absents soit chez *Neisseria gonorrhoeae* (désignée ci-après par Ng), soit chez Neisseria Pactamica (désignée ci-après par Nl) à l'exception des gènes impliqués dans la biosynthèse de la capsule polysaccharidique, *frpA*, *frpC*, *opc*, *por*A, rotamase, de la séquence IC1106, des IgA protéases, de la pilline, de pilC, des protéines qui lient la transferrine et des protéines d'opacité..

2/ ADN selon la revendication 1, caractérisés en ce qu'ils sont présents chez Nm, mais absents chez Ng.

3/ ADN selon la revendication 2, caractérisés en ce qu'ils comprennent une ou plusieurs séquence(s) telle(s) que présente(s) sur le chromosome de Nm Z2491 entre *tuf*A et *pilT*, ou région 1 du chromosome, et/ou la ou les séquence(s) nucléotidique(s) capable(s) de s'hybrider avec la ou lesdites séquences.

4/ ADN selon la revendication 2, caractérisés en ce qu'ils comprennent une ou plusieurs séquence(s) telle(s) que présente(s) sur le chromosome de Nm Z2491 entre *pilQ* et *λ740*, ou région 2 du chromosome, et/ou la ou les séquence(s) nucléotidique(s) capable(s) de s'hybrider avec la ou lesdites séquences.

5/ ADN selon la revendication 2, caractérisés en ce qu'ils comprennent une ou plusieurs séquence(s) telle(s) que présente(s) sur le chromosome de Nm Z2491 entre *argF* et *opaB*, ou région 3 du chromosome, et/ou la ou les séquence(s) capable(s) de s'hybrider avec la ou lesdites séquences.

6/ ADN selon la revendication 3, caractérisés en ce que leur séquence correspond, pour tout ou partie, à SEQ ID n° 9, 13, 22 ou 30, et/ou à toute séquence se situant à plus ou moins 20 kb de ces SEQ ID sur le

chromosome d'une souche de Nm, et/ou est capable de s'hybrider avec au moins un fragment de l'une quelconque de ces séquences.

7/ ADN selon la revendication 4, caractérisés en ce que leur séquence correspond pour, tout ou partie, à SEQ ID n° 1, 2, 4, 6, 7, 10, 15, 31 ou 34, et/ou, à toute séquence se situant à plus ou moins 20 kb de ces SEQ ID sur le chromosome d'une souche de Nm, et/ou est capable de s'hybrider avec au moins un fragment de l'une quelconque de ces séquences.

8/ ADN selon la revendication 4, caractérisés en ce qu'il s'agit de tout ou partie de la séquence d'ADN SEQ ID N°36 ou de séquences correspondant aux cadres ouverts de lecture SEQ ID N°37, SEQ ID N°38, SEQ ID N°39, SEQ ID N°40, SEQ ID N°41, SEQ ID N°42, SEQ ID N°43, SEQ ID N°44, SEQ ID N°45 et/ou à toute séquence se situant à plus ou moins 20 kb de ces SEQ ID sur le chromosome d'une souche de Nm, et/ou est capable de s'hybrider avec au moins un fragment de l'une quelconque de ces séquences.

9/ ADN selon la revendication 5, caractérisés en ce que leur séquence correspond, pour tout ou partie, à SEQ ID n° 8, 21, 23, 25, 26, 28, 29, 32 ou 35, et/ou, à toute séquence se situant à plus ou moins 20 kb de ces SEQ ID sur le chromosome d'une souche de Nm, et/ou est capable de s'hybrider avec au moins un fragment de l'une quelconque de ces séquences.

10/ ADN selon la revendication 2, caractérisés en ce que leur séquence correspond, pour tout ou partie, à SEQ ID n° 3, 5, 11, 12, 14, 16, 18, 19, 20, 24, 27 ou 33, et/ou à toute séquence se situant à plus ou moins 20 kb de ces SEQ ID sur le chromosome d'une souche de Nm, et/ou, est capable de s'hybrider avec au moins un fragment de l'une quelconque de ces séquences.

11/ ADN selon la revendication 1, caractérisé en ce qu'ils sont communs avec ceux de Ng, mais sont absents de chez N1.

12/ ADN selon la revendication 11, caractérisé en ce qu'ils comprennent une ou plusieurs séquence(s) telle(s) que présente(s) sur le chromosome de Nm Z2491 entre arg J et reg F, ou région 4 du chromosome et/ou la ou les séquence(s) nucléotique(s) capable(s) de s'hybrider avec la ou lesdites séquences.

13/ ADN selon la revendication 11, caractérisés en ce qu'ils comprennent une ou plusieurs séquence(s) telle(s) que présente(s) sur le chromosome de Nm Z2491 entre le marqueur lambda 375 à pen A, ou région 5 du chromosome et/ou la ou les séquence(s) nucléotique(s) capable(s) de s'hybrider avec la ou lesdites séquences.

14/ ADN selon l'une quelconque des revendications précédentes, caractérisé en ce qu'il code pour une protéine exportée au-delà de la membrane cytoplasmique.

15/ ADN selon l'une quelconque des revendications 1 à 14, caractérisés en ce que tout ou partie de leur séquence correspond à une région conservée au sein de l'espèce Nm.

16/ ADN selon l'une quelconque des revendications 1 à 15, caractérisé en ce qu'il est inséré dans un vecteur de transfert ou d'expression tel que cosmide, plasmide ou bactériophage.

17/ Cellule hôte, plus particulièrement cellule bactérienne ou cellule de Nm, transformée par l'insertion d'au moins un ADN selon l'une quelconque des revendications 1 à 15.

18/Cellule comportant des gènes ou des fragments de gènes spécifiques de Nm, plus particulièrement cellule bactérienne, ou cellule de Nm, dont le chromosome est délété d'au moins un ADN selon

l'une quelconque des revendications 1 à 15, en particulier d'un ADN responsable de la pathogénicité.

19/ ARN, caractérisés en ce que leur séquence correspond pour tout ou partie à la transcription d'au moins une séquence ou fragment de séquence d'ADN selon l'une quelconque des revendications 1 à 15.

20/ Acides nucléiques anti-sens, caractérisés en ce que leur séquence correspond à l'anti-sens d'au moins une séquence nucléotidique selon l'une quelconque des revendications 1 à 15 ou 19, ou d'un fragment d'une telle séquence, et qu'ils portent, le cas échéant, au moins une substitution chimique telle qu'un groupe méthyle et/ou un groupe glycosyle.

21/ Polypeptides, caractérisés en ce qu'ils présentent un enchaînement d'acides aminés correspondant à tout ou partie d'une séquence telle que codée par les acides nucléiques définis dans l'une quelconque des revendications 1 à 15 ou 19, ou tel que déduit des séquences de ces acides nucléiques, avec, le cas échéant, des modifications par rapport aux séquences codées ou déduites dès lors que ces modifications n'altèrent pas les propriétés biochimiques telles qu'observées chez le polypeptide natif.

22/ Peptides selon la revendication 21, caractérisés en ce qu'il s'agit de peptides exportés au-delà de la membrane cytoplasmique, plus spécialement de peptides correspondant à tout ou partie de ceux codés par un ADN selon la revendication 14.

23/ Anticorps, caractérisés en ce qu'il s'agit d'anticorps polyclonaux ou monoclonaux dirigés contre au moins un épitope d'un peptide selon la revendication 20 ou 21, ou de fragments de ces anticorps, plus particulièrement les fragments Fv, Fab, Fab'2, ou encore d'anti-anticorps capables de reconnaître, selon une

réaction de type antigène-anticorps, lesdits anticorps ou leurs fragments.

24/ Procédé d'obtention de banques d'ADN *Neisseria meningitidis*-spécifiques, comprenant :

- le mélange de deux populations d'ADN,
- la réalisation d'au moins une itération d'hybridation-amplification soustractive, et
- la récupération du ou des ADN souhaités, suivie le cas échéant de leur purification avec l'élimination des séquences redondantes.

25/ Procédé selon la revendication 24, caractérisés en ce que pour obtenir une banque Nm spécifique par rapport à Ng

- on mélange deux populations d'ADN provenant respectivement d'une souche de *Neisseria meningitidis*, ou souche de référence, pour laquelle la banque spécifique doit être constituée, et d'une souche de *Neisseria gonorrhoeae*, ou souche de soustraction, les séquences d'ADN de ces souches étant telles qu'obtenues par
  - . cisaillement aléatoire de l'ADN chromosomique de la souche de soustraction, notamment par passages répétés à travers une seringue, et
  - . clivage de l'ADN chromosomique de la souche de référence, de préférence par une enzyme de restriction produisant des fragments de taille inférieure à 1kb environ, et que pour obtenir une banque d'ADN communs entre Nm et Ng, mais spécifiques par rapport à Nl, on constitue trois banques différentes, dont deux par digestion de l'ADN chromosomique de Nm par *MBoI* et *Tsp5091*, et la troisième, par digestion de l'ADN chromosomique de Nm avec *MspI*, on opère deux séries de soustraction et on récupère les ADN présentant la spécificité recherchée.

26/ Banques de clones d'ADN telles qu'obtenues par mise en oeuvre du procédé selon la revendication 24 ou 25.

27/ Application du procédé selon la revendication 24 pour l'obtention de banques d'ADN spécifiques d'une cellule donnée ou d'un variant donné d'une même espèce de cellule, dès lors qu'il existe une autre espèce ou un autre variant proche génomiquement, et exprimant des pouvoirs pathogènes différents, en particulier de banques d'ADN spécifiques de cryptocoques, d'Haemophilus, de pneumocoques ou encore d'Escherichia.

28/ Méthode de diagnostic d'une infection méningococcique, et plus particulièrement de la méningite méningococcique, par mise en évidence de la présence de *Neisseria meningitidis* dans un échantillon biologique caractérisée en ce qu'elle comprend les étapes de :

- mise en contact d'un échantillon biologique à analyser, avec un réactif élaboré à partir d'au moins un acide nucléique tel que défini dans l'une des revendications 1 à 15, ou 19, le cas échéant sous forme de sonde nucléotidique, ou d'amorce, ou en variante à partir d'au moins un anticorps, ou un fragment d'anticorps, tel que défini dans la revendication 23, dans des conditions permettant respectivement une hybridation ou une réaction de type antigène-anticorps, et

- révélation du produit de réaction éventuellement formé.

29/ Méthode de diagnostic d'une réaction immunitaire spécifique de l'infection méningococcique, caractérisée en ce qu'elle comprend les étapes de :

- mise en contact d'un échantillon biologique à analyser avec au moins un polypeptide selon l'une quelconque des revendications 21 ou 22 ou d'un anti-anticorps selon la revendication 23, ou d'un fragment de

celui-ci, ces produits étant, le cas échéant, marqués dans des conditions permettant la réalisation d'une réaction de type antigène-anticorps, et

- révélation du produit de réaction éventuellement formé.

30/ Kits pour la mise en oeuvre d'une méthode selon l'une quelconque des revendications 28 ou 29, caractérisés en ce qu'ils comportent :

- au moins un réactif tel que défini dans la revendication 28 ou 29, à savoir de type acide nucléique, anticorps ou peptide,

- les produits, notamment marqueurs ou tampons, permettant la réalisation de la réaction d'hybridation nucléotidique ou de la réaction immunologique visée, ainsi qu'une notice d'utilisation.

31/ Composition vaccinale incluant dans son spectre, en particulier en association avec au moins un vaccin pour l'enfance, une prophylaxie à visée anti-méningococcique, et destinée à prévenir toute forme d'infection par *Neisseria meningitidis*, caractérisée en ce qu'elle comprend, en association avec un/des véhicule(s) physiologiquement acceptable(s), une quantité efficace :

- de peptide selon la revendication 21 ou 22, ou

- d'anticorps ou de fragment d'anti-anticorps selon la revendication 23,

ce matériel étant éventuellement conjugué, afin de renforcer son immunogénicité, à une molécule porteuse telle que protéine de polyovirus, toxine tétanique, protéine issue de la région hypervariable d'une piline.

32/ Composition vaccinale incluant dans son spectre, en particulier en association avec au moins un vaccin pour l'enfance, une prophylaxie à visée anti-méningococcique, et destinée à prévenir toute forme

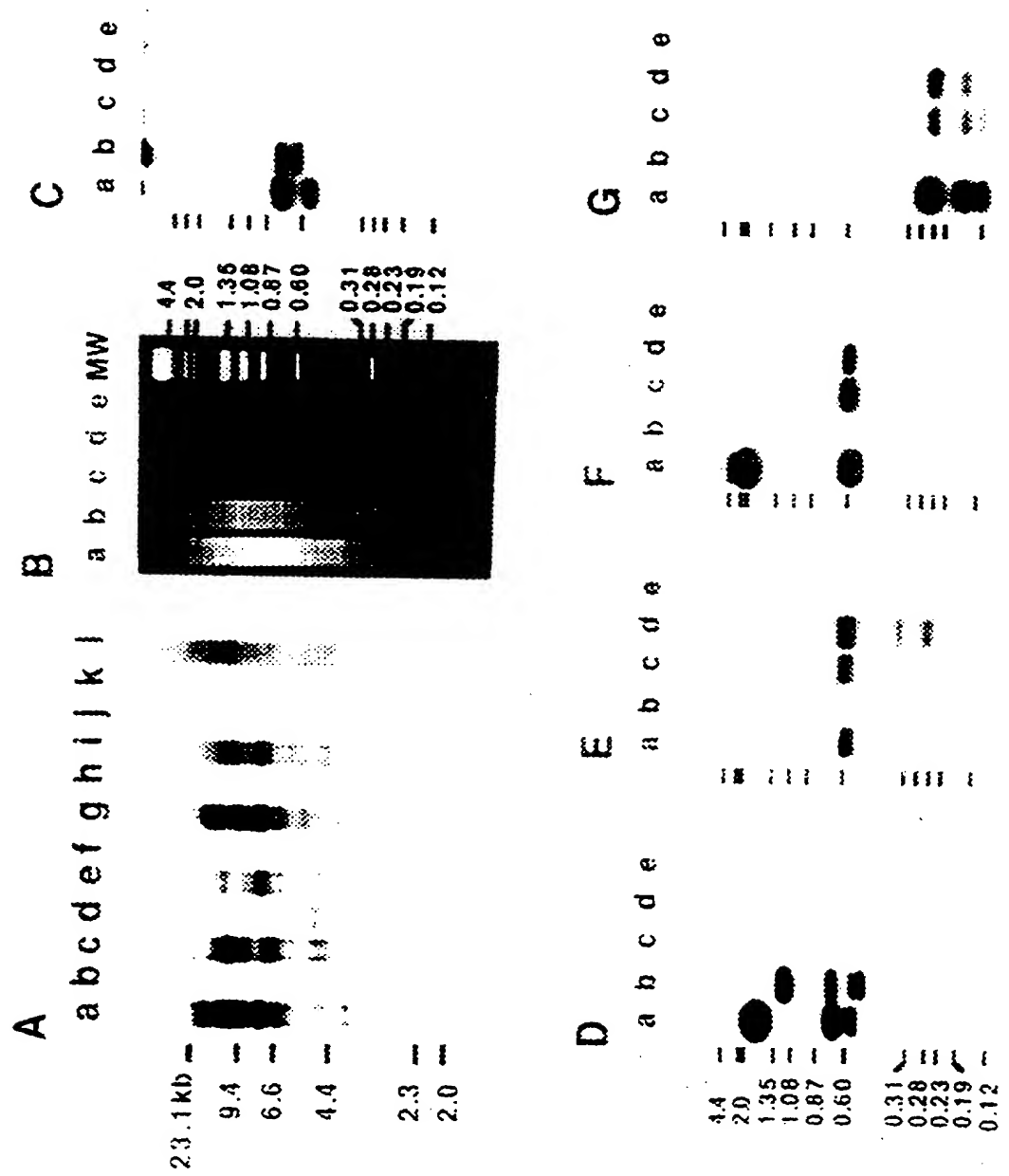
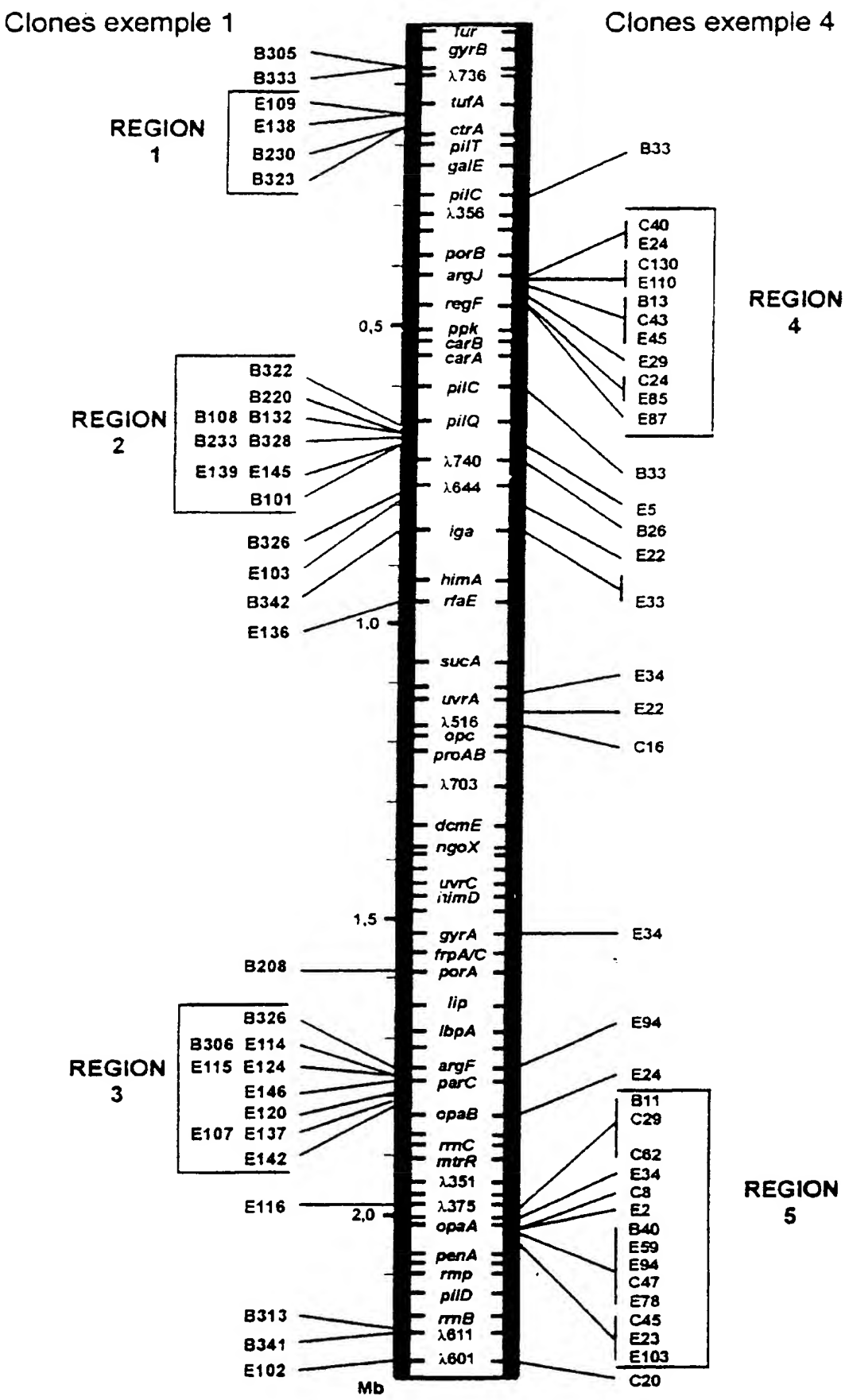


Figure 2



FEUILLE DE REMPLACEMENT (REGLE 26)

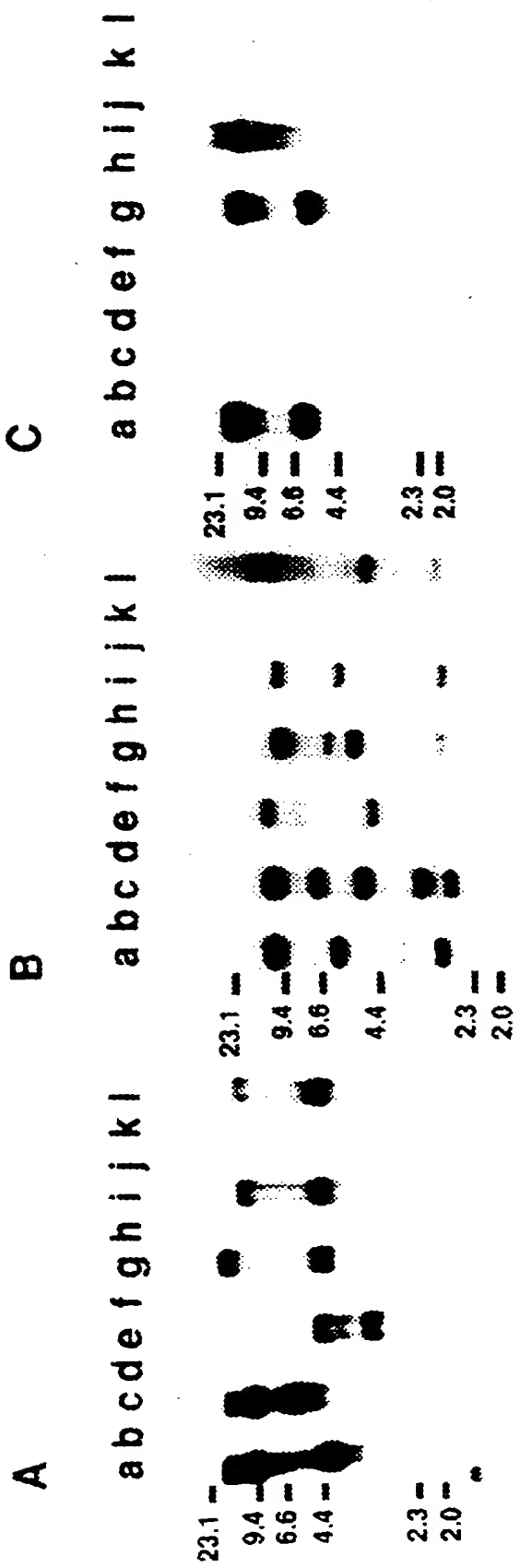
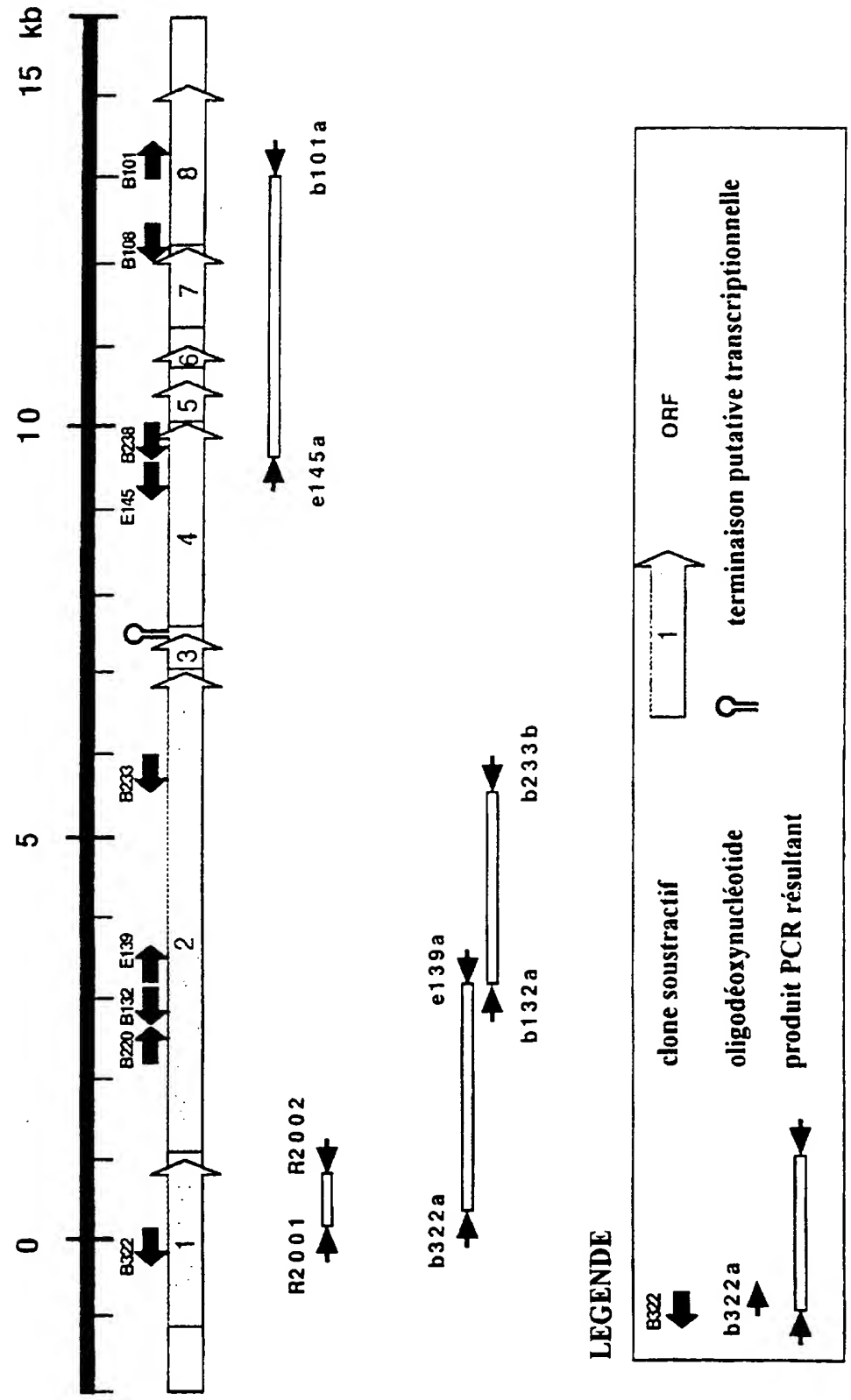


Figure 4



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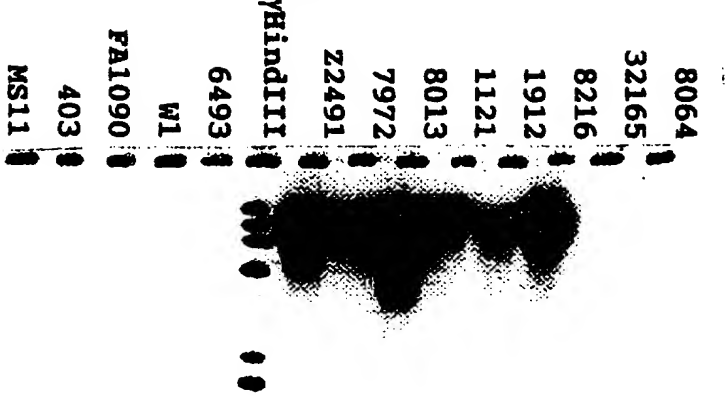


Figure 6

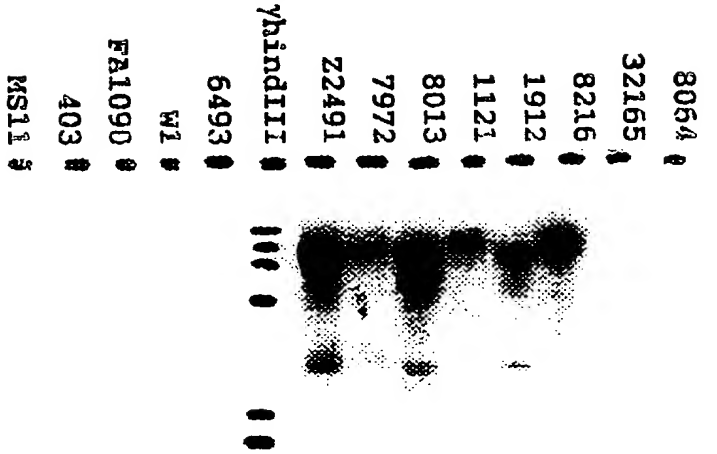


Figure 7

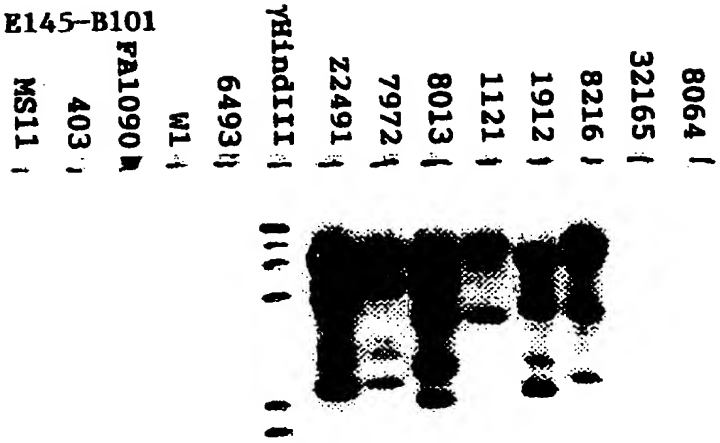


Figure 8A

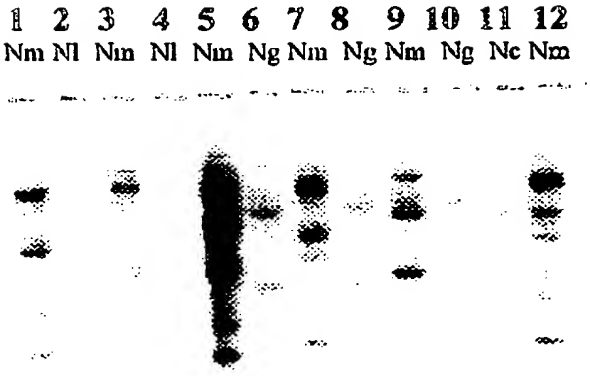


Figure 8B

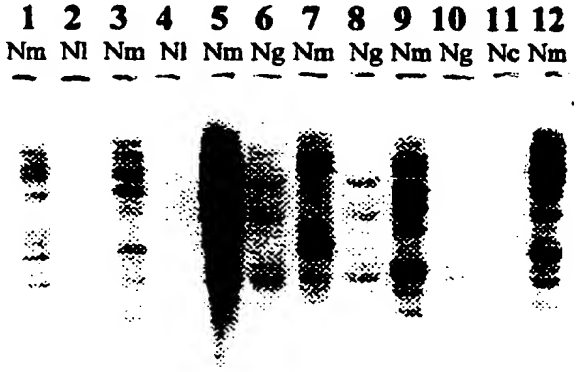


Figure 8C

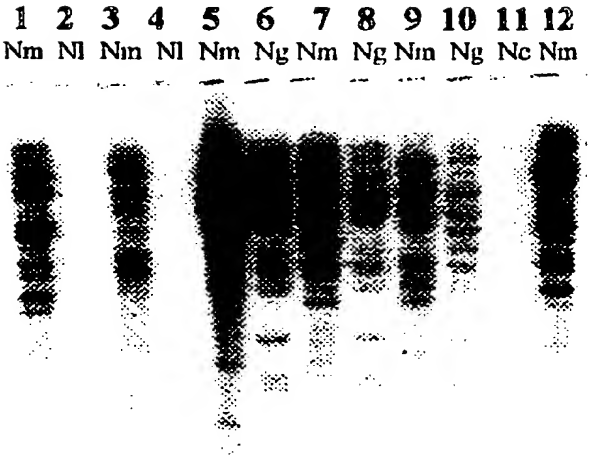


Figure 9

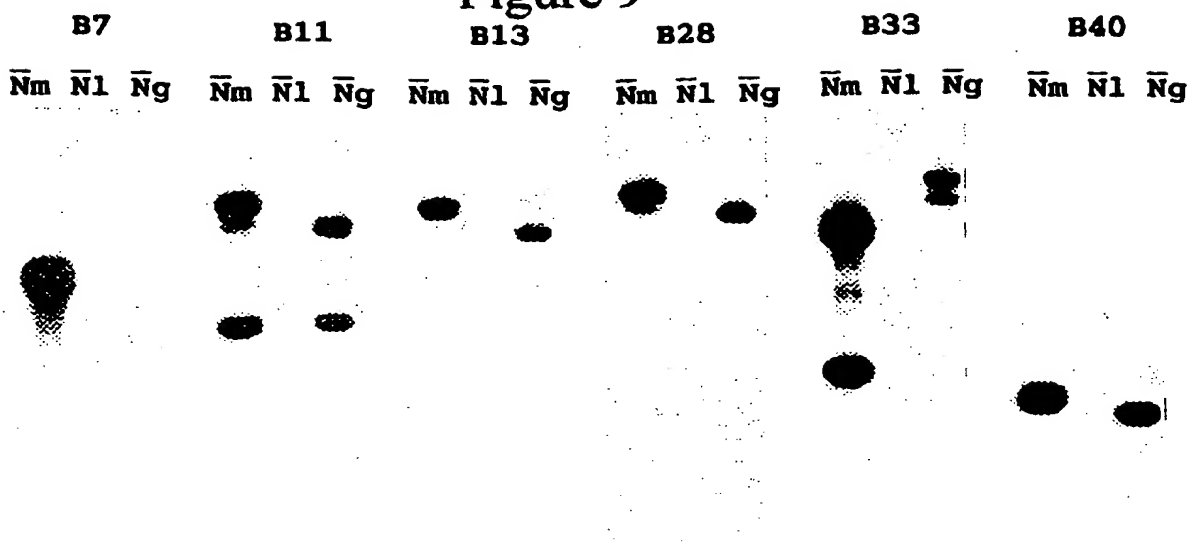


Figure 10

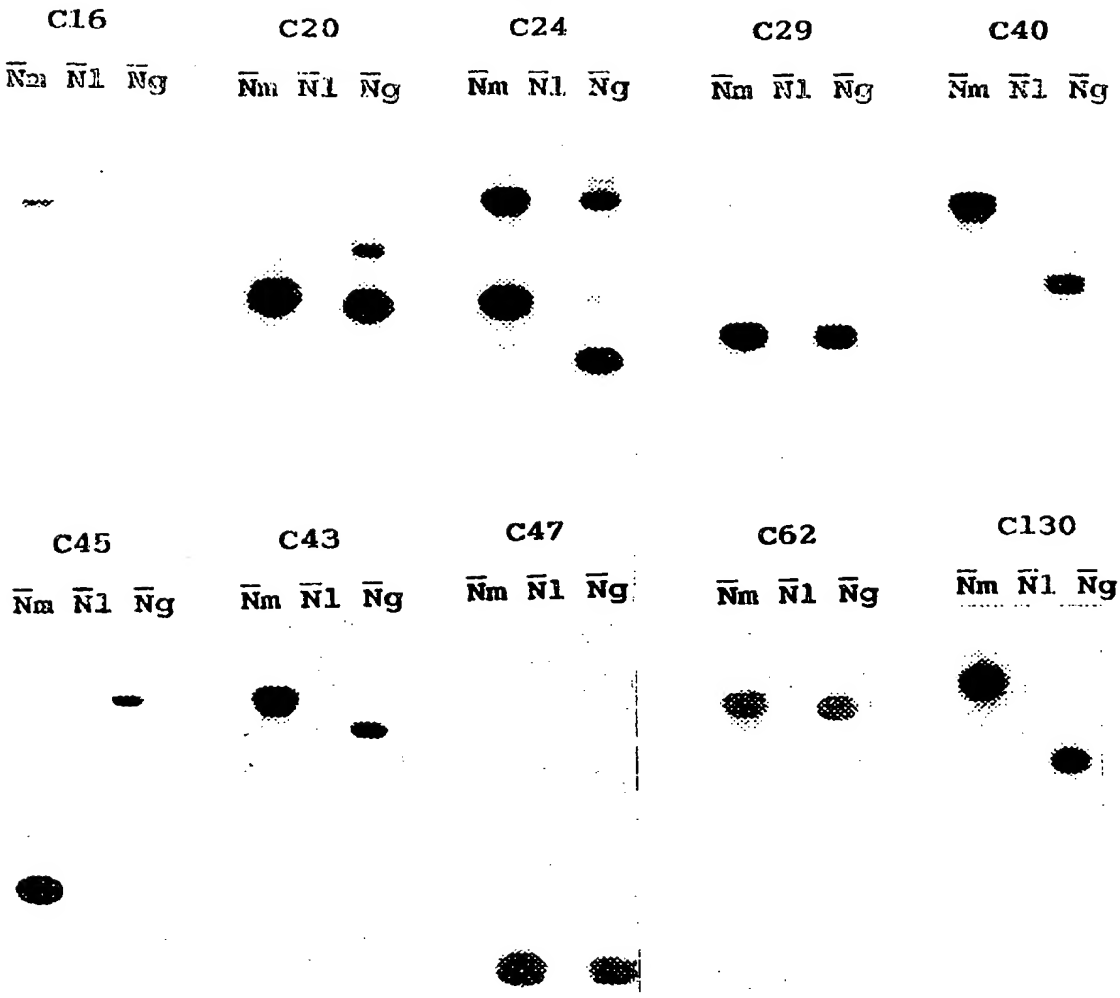
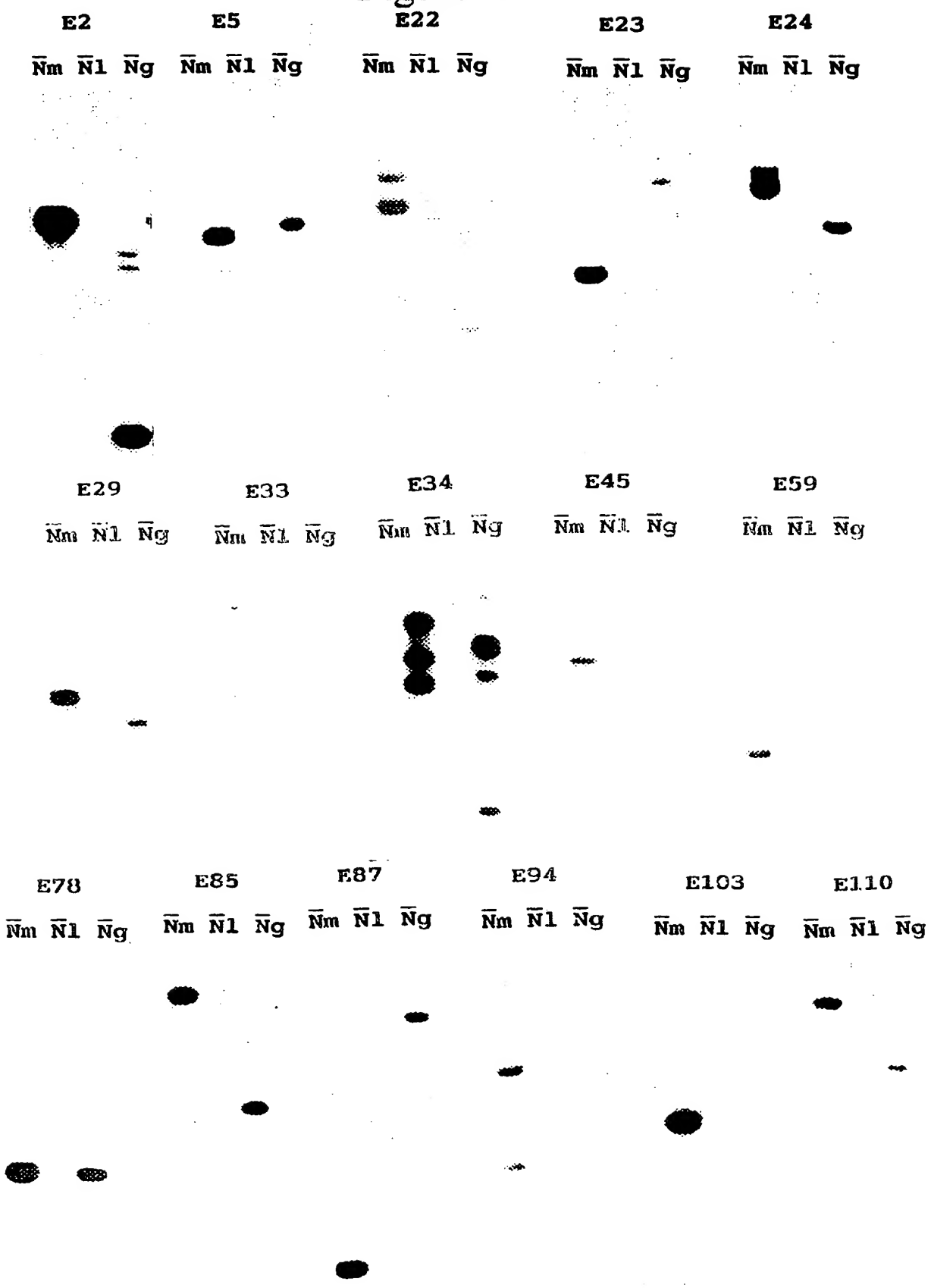


Figure 11



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Bureau international

DEMANDE INTERNATIONALE PUBLIEE EN VERTU DU TRAITE DE COOPERATION EN MATIERE DE BREVETS (PCT)

(51) Classification internationale des brevets 6 : C12N 15/31, C07K 14/22, 16/12, A61K 39/095, C12Q 1/68, G01N 33/53 — A3

(11) Numéro de publication internationale: **WO 98/02547**

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96/08768 12 juillet 1996 (12.07.96) FR

(71) Déposants *(pour tous les Etats désignés sauf US)*: INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM) [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cedex 13 (FR). MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V., BERLIN [DE/DE]; Hofgartenstrasse 2, D-80539 Münich (DE). SMITHKLINE BEECHAM [GB/GB]; New Horizons Court, Brentford TW8 9EP (GB).

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(74) Mandataires: PEAUCELLE, Chantal etc.; Cabinet Armengaud Ainé, 3, avenue Bugeaud, F-75116 Paris (FR).

(81) Etats désignés: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, brevet ARIPO (GH, KE, LS, MW, SD, SZ, UG, ZW), brevet eurasien (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), brevet européen (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Publiée
Avec rapport de recherche internationale.
Avant l'expiration du délai prévu pour la modification des revendications, sera republiée si de telles modifications sont reçues.

(88) Date de publication du rapport de recherche internationale: 9 avril 1998 (09.04.98)

(54) Title: DNA AND SPECIFIC PROTEINS OR PEPTIDES OF THE *NEISSERIA MENINGITIDIS* SPECIES BACTERIA, METHOD FOR OBTAINING THEM AND THEIR BIOLOGICAL APPLICATIONS

(54) Titre: ADN ET PROTEINES OU PEPTIDES SPECIFIQUES DES BACTERIES DE L'ESPECE *NEISSERIA MENINGITIDIS*, LEURS PROCEDES D'OBTENTION ET LEURS APPLICATIONS BIOLOGIQUES

(57) Abstract

The DNA of the invention are characterised in that they concern the whole or part of genes, with their reading frame, to be found in *Neisseria meningitidis*, but not in *Neisseria gonorrhoeae*, or in *Neisseria lactamica* except the genes involved in the biosynthesis of the polysaccharide capsule, *frp*A, *frp*C, *opc*, *por*A, rotamase the sequence IC1106, IgA protease, pilline, pilC, transferrin binding proteins and opacity proteins. The invention also concerns the polypeptides corresponding to these DNA and the antibodies directed against these polypeptides. It is applicable in the prevention and the detection of meningococcus induced infections and meningitis.

(57) Abrégé

Les ADN de l'invention sont caractérisés en ce qu'il s'agit de tout ou partie de gènes, avec leur phase de lecture, présents chez *Neisseria meningitidis*, mais absents soit chez *Neisseria gonorrhoeae*, soit chez *Neisseria lactamica* à l'exception des gènes impliqués dans la biosynthèse de la capsule polysaccharidique, *frp*A, *frp*C, *opc*, *por*A, rotamase, de la séquence IC1106, des IgA protéases, de la pilline, de pilC, des protéines qui lient la transferrine et des protéines d'opacité. L'invention vise également les polypeptides correspondant à ces ADN et les anticorps dirigés contre ces polypeptides. Applications à la prévention et à la détection d'infections à méningocoques et de méningites.

UNIQUEMENT A TITRE D'INFORMATION

Codes utilisés pour identifier les Etats parties au PCT, sur les pages de couverture des brochures publiant des demandes internationales en vertu du PCT.

AL	Albanie
AM	Arménie
AT	Autriche
AU	Australie
AZ	Azerbaïdjan
BA	Bosnie-Herzégovine
BB	Barbade
BE	Belgique
BF	Burkina Faso
BG	Bulgarie
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BR	Brésil
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CA	Canada
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CG	Congo
CH	Suisse
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CM	Cameroun
CN	Chine
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CZ	République tchèque
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HU	Hongrie
IE	Irlande
IL	Israël
IS	Islande
IT	Italie
JP	Japon
KE	Kenya
KG	Kirghizistan
KP	République populaire démocratique de Corée
KR	République de Corée
KZ	Kazakstan
LC	Sainte-Lucie
LI	Liechtenstein
LK	Sri Lanka
LR	Libéria
LS	Lesotho
LT	Lituanie
LU	Luxembourg
LV	Lettonie
MC	Monaco
MD	République de Moldova
MG	Madagascar
MK	Ex-République yougoslave de Macédoine
ML	Mali
MN	Mongolie
MR	Mauritanie
MW	Malawi
MX	Mexique
NE	Niger
NL	Pays-Bas
NO	Norvège
NZ	Nouvelle-Zélande
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SK	Slovaquie
SN	Sénégal
SZ	Swaziland
TD	Tchad
TG	Togo
TJ	Tadjikistan
TM	Turkménistan
TR	Turquie
TT	Trinité-et-Tobago
UA	Ukraine
UG	Ouganda
US	Etats-Unis d'Amérique
UZ	Ouzbékistan
VN	Viet Nam
YU	Yougoslavie
ZW	Zimbabwe

BNSDOCID: <WO___9802547A3_I_>

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FR 97/01295

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C07K14/22 C07K16/12 A61K39/095 C12Q1/68
G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHOU J ET AL: "Sequence diversity within the argF, fbp and recA genes of natural isolates of Neisseria meningitidis: interspecies recombination within the argF gene." MOL MICROBIOL, AUG 1992, 6 (15) P2135-46, ENGLAND, XP000645119	1,2, 14-22
Y	see the whole document	23,28-32
A		11
Y	WO 94 05703 A (GLOBAL TEK INC) 17 March 1994 see page 16 - page 26; table 3	23,28-32

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1998	27.02.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DEVI SJ ET AL: "Antibodies to poly[(2----8)-alpha-N-acetylneuraminic acid] and poly[(2----9)-alpha-N-acetylneuraminic acid] are elicited by immunization of mice with Escherichia coli K92 conjugates: potential vaccines for groups B and C meningococci and E. coli K1." PROC NATL ACAD SCI U S A, AUG 15 1991, 88 (16) P7175-9, UNITED STATES, XP002044636 see the whole document	31,32
Y	WOLFF K ET AL: "Identification and characterization of specific sequences encoding pathogenicity associated proteins in the genome of commensal Neisseria species." FEMS MICROBIOL LETT, JAN 15 1995, 125 (2-3) P255-63, NETHERLANDS, XP002044637 see abstract; figure 1; table 1	1-3,11, 14-23, 28-32
A	PETERING H ET AL: "Genes associated with meningococcal capsule complex are also found in Neisseria gonorrhoeae." J BACTERIOL, JUN 1996, 178 (11) P3342-5, UNITED STATES, XP002044638 see abstract; figures 4,5,7	1,2
A	FROSCH M ET AL: "Evidence for a common molecular origin of the capsule gene loci in gram-negative bacteria expressing group II capsular polysaccharides." MOL MICROBIOL, MAY 1991, 5 (5) P1251-63, ENGLAND, XP000647762 see the whole document	1-3,6, 14-22
A	FROSCH M ET AL: "Phospholipid substitution of capsular polysaccharides and mechanisms of capsule formation in Neisseria meningitidis." MOL MICROBIOL, MAY 1993, 8 (3) P483-93, ENGLAND, XP000647767 see the whole document	1-3,6, 14-22
A	FROSCH M ET AL: "CONSERVED OUTER-MEMBRANE PROTEIN OF NEISSERIA-MENINGITIDIS INVOLVED IN CAPSULE EXPRESSION" INFECTION AND IMMUNITY, 1992, 60, 798-803, XP002044642 see the whole document	23,28-32
A	EP 0 452 596 A (INNOGENETICS NV) 23 October 1991 see example 1	1,2, 28-30

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 88 03957 A (GEN PROBE INC) 2 June 1988 see example 21	1,2, 28-30
A	EP 0 337 896 A (INNOGENETICS NV) 18 October 1989 see page 17, paragraph 2 - page 24, line 55	1,2, 28-30
X	STRATHDEE CA ET AL: "IDENTIFICATION OF EPIDEMIOLOGIC MARKERS FOR NEISSERIA-MENINGITIDIS USING DIFFERENCE ANALYSIS" GENE, 1995, 166, 105-110, XP002044641	24,26,27
Y	see the whole document	25
Y	SCHUTTE M ET AL: "Isolation of YAC insert sequences by representational difference analysis" NUCLEIC ACIDS RESEARCH, 23 (20). 1995. 4127-4133., XP002052562 see abstract	25
Y	LAUERMAN LH ET AL: "Avian mycoplasma identification using polymerase chain reaction amplicon and restriction fragment length polymorphism analysis." AVIAN DIS, OCT-DEC 1995, 39 (4) P804-11, UNITED STATES, XP002052563 see abstract	25
Y	WO 90 15621 A (STATENS SERUMINSTITUT) 27 December 1990 see page 55, line 36 - page 56, line 13	25
A	LISITSYN N ET AL: "Cloning the differences between two complex genomes." SCIENCE, FEB 12 1993, 259 (5097) P946-51, UNITED STATES, XP002052564 see the whole document	25-27
P,X	TINSLEY CR ET AL: "Analysis of the genetic differences between Neisseria meningitidis and Neisseria gonorrhoeae: two closely related bacteria expressing two different pathogenicities." PROC NATL ACAD SCI U S A, OCT 1 1996, 93 (20) P11109-14, UNITED STATES, XP002028346 see the whole document	1-3,6, 14-23, 28-32

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FR 97/01295

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

SEE SUPPLEMENTARY SHEET

1 ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims : 2-10 and 1,14-23,28-32 partly

DNAs characterized in that they comprise one or more sequences such as those present in Neisseria meningitidis but absent from Neisseria gonnorheae, host cell, RNA, antisense nucleic acids, polypeptides, antibodies, diagnostic method, diagnostic kit and vaccinal composition thereof.

2. Claims: 11-13 and 1,14-23, 28-32 partly
DNAs characterized in that they comprise one or more sequences such as those present in neisseria meningitidis but absent from Neisseria lactamica, host cell, RNA, antisense nucleic acids, polypeptides, antibodies, diagnostic method, diagnostic kit and vaccinal composition thereof.

3. Claims: 24-27
Method of obtaining specific DNA banks neisseria meningitidis comprising the mixture of a stock of Neisseria meningitidis with a stock of Neisseira gonnorheae, i.e. Neisseria lactamica, DNA clone banks and the applications thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/FR 97/01295

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9405703 A	17-03-94	AU 5093693 A	29-03-94
		CA 2122630 A	17-03-94
		EP 0625165 A	23-11-94
EP 0452596 A	23-10-91	AT 128189 T	15-10-95
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		ES 2080945 T	16-02-96
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		AU 1041988 A	16-06-88
		DK 413788 A	23-09-88
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		JP 1503356 T	16-11-89
		KR 9511719 B	09-10-95
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		US 5677129 A	14-10-97
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		US 5693469 A	02-12-97
		US 5679520 A	21-10-97
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EP 0337896 A	18-10-89	AU 615286 B	26-09-91
		AU 3300489 A	19-10-89
		CA 1339261 A	12-08-97
		DE 68907772 T	04-11-93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0337896 A		ES 2058566 T	01-11-94
		JP 2203800 A	13-08-90
WO 9015621 A	27-12-90	AU 5851390 A	08-01-91

RAPPORT DE RECHERCHE INTERNATIONALE

Demande Internationale No
PCT/FR 97/01295

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
CIB 6 C12N15/31 C07K14/22 C07K16/12 A61K39/095 C12Q1/68
G01N33/53

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
CIB 6 C07K C12Q

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si cela est réalisable, termes de recherche utilisés)

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie °	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X	ZHOU J ET AL: "Sequence diversity within the argF, fbp and recA genes of natural isolates of Neisseria meningitidis: interspecies recombination within the argF gene." MOL MICROBIOL, AUG 1992, 6 (15) P2135-46, ENGLAND, XP000645119	1,2, 14-22
Y	voir le document en entier	23,28-32
A		11

Y	WO 94 05703 A (GLOBAL TEK INC) 17 mars 1994 voir page 16 - page 26; tableau 3	23,28-32

	-/--	

[X] Voir la suite du cadre C pour la fin de la liste des documents

[X] Les documents de familles de brevets sont indiqués en annexe

° Catégories spéciales de documents cités:

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document antérieur, mais publié à la date de dépôt international ou après cette date

"L" document pouvant jeter un doute sur une revendication de priorité ou cité pour déterminer la date de publication d'une autre citation ou pour une raison spéciale (telle qu'indiquée)

"O" document se référant à une divulgation orale, à un usage, à une exposition ou tous autres moyens

"P" document publié avant la date de dépôt international, mais postérieurement à la date de priorité revendiquée

"T" document ultérieur publié après la date de dépôt international ou la date de priorité et n'appartenenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive par rapport au document considéré isolément

"Y" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant évidente pour une personne du métier

"&" document qui fait partie de la même famille de brevets

6

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
29 janvier 1998	27.02.98

Nom et adresse postale de l'administration chargée de la recherche internationale	Fonctionnaire autorisé
Office Européen des Brevets, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

Formulaire PCT/ISA/210 (deuxième feuille) (juillet 1992)

C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie *	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
Y	DEVI SJ ET AL: "Antibodies to poly[(2----8)-alpha-N-acetylneuraminic acid] and poly[(2----9)-alpha-N-acetylneuraminic acid] are elicited by immunization of mice with Escherichia coli K92 conjugates: potential vaccines for groups B and C meningococci and E. coli K1." PROC NATL ACAD SCI U S A, AUG 15 1991, 88 (16) P7175-9, UNITED STATES, XP002044636 voir le document en entier	31,32
A	WOLFF K ET AL: "Identification and characterization of specific sequences encoding pathogenicity associated proteins in the genome of commensal Neisseria species." FEMS MICROBIOL LETT, JAN 15 1995, 125 (2-3) P255-63, NETHERLANDS, XP002044637 voir abrégé; figure 1; tableau 1	1-3,11, 14-23, 28-32
A	PETERING H ET AL: "Genes associated with meningococcal capsule complex are also found in Neisseria gonorrhoeae." J BACTERIOL, JUN 1996, 178 (11) P3342-5, UNITED STATES, XP002044638 voir abrégé; figures 4,5,7	1,2
A	FROSCH M ET AL: "Evidence for a common molecular origin of the capsule gene loci in gram-negative bacteria expressing group II capsular polysaccharides." MOL MICROBIOL, MAY 1991, 5 (5) P1251-63, ENGLAND, XP000647762 voir le document en entier	1-3,6, 14-22
A	FROSCH M ET AL: "Phospholipid substitution of capsular polysaccharides and mechanisms of capsule formation in Neisseria meningitidis." MOL MICROBIOL, MAY 1993, 8 (3) P483-93, ENGLAND, XP000647767 voir le document en entier	1-3,6, 14-22
A	FROSCH M ET AL: "CONSERVED OUTER-MEMBRANE PROTEIN OF NEISSERIA-MENINGITIDIS INVOLVED IN CAPSULE EXPRESSION" INFECTION AND IMMUNITY, 1992, 60, 798-803, XP002044642 voir le document en entier	23,28-32
A	EP 0 452 596 A (INNOGENETICS NV) 23 octobre 1991 voir exemple 1	1,2, 28-30

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6
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C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie *	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
A	WO 88 03957 A (GEN PROBE INC) 2 juin 1988 voir exemple 21	1,2, 28-30
A	EP 0 337 896 A (INNOGENETICS NV) 18 octobre 1989 voir page 17, alinéa 2 - page 24, ligne 55	1,2, 28-30
X	STRATHDEE CA ET AL: "IDENTIFICATION OF EPIDEMIOLOGIC MARKERS FOR NEISSERIA-MENINGITIDIS USING DIFFERENCE ANALYSIS" GENE, 1995, 166, 105-110, XP002044641	24,26,27
Y	voir le document en entier	25
Y	SCHUTTE M ET AL: "Isolation of YAC insert sequences by representational difference analysis" NUCLEIC ACIDS RESEARCH, 23 (20). 1995. 4127-4133., XP002052562 voir abrégé	25
Y	LAUERMAN LH ET AL: "Avian mycoplasma identification using polymerase chain reaction amplicon and restriction fragment length polymorphism analysis." AVIAN DIS, OCT-DEC 1995, 39 (4) P804-11, UNITED STATES, XP002052563 voir abrégé	25
Y	WO 90 15621 A (STATENS SERUMINSTITUT) 27 décembre 1990 voir page 55, ligne 36 - page 56, ligne 13	25
A	LISITSYN N ET AL: "Cloning the differences between two complex genomes." SCIENCE, FEB 12 1993, 259 (5097) P946-51, UNITED STATES, XP002052564 voir le document en entier	25-27
P,X	TINSLEY CR ET AL: "Analysis of the genetic differences between Neisseria meningitidis and Neisseria gonorrhoeae: two closely related bacteria expressing two different pathogenicities." PROC NATL ACAD SCI U S A, OCT 1 1996, 93 (20) P11109-14, UNITED STATES, XP002028346 voir le document en entier	1-3,6, 14-23, 28-32

6

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale n° PCT/FR 97/01295

Cadre I Observations - lorsqu'il a été estimé que certaines revendications ne pouvaient pas faire l'objet d'une recherche (suite du point 1 de la première feuille)

Conformément à l'article 17.2)a), certaines revendications n'ont pas fait l'objet d'une recherche pour les motifs suivants:

1. ☐ Les revendications n°s
se rapportent à un objet à l'égard duquel l'administration n'est pas tenue de procéder à la recherche, à savoir:

2. ☐ Les revendications n°s
se rapportent à des parties de la demande internationale qui ne remplissent pas suffisamment les conditions prescrites pour qu'une recherche significative puisse être effectuée, en particulier:

3. ☐ Les revendications n°s
sont des revendications dépendantes et ne sont pas rédigées conformément aux dispositions de la deuxième et de la troisième phrases de la règle 6.4.a).

Cadre II Observations - lorsqu'il y a absence d'unité de l'invention (suite du point 2 de la première feuille)

L'administration chargée de la recherche internationale a trouvé plusieurs inventions dans la demande internationale, à savoir:

voir feuille supplémentaire

1. ☒ Comme toutes les taxes additionnelles ont été payées dans les délais par le déposant, le présent rapport de recherche internationale porte sur toutes les revendications pouvant faire l'objet d'une recherche.

2. ☐ Comme toutes les recherches portant sur les revendications qui s'y prêtaient ont pu être effectuées sans effort particulier justifiant une taxe additionnelle, l'administration n'a sollicité le paiement d'aucune taxe de cette nature.

3. ☐ Comme une partie seulement des taxes additionnelles demandées a été payée dans les délais par le déposant, le présent rapport de recherche internationale ne porte que sur les revendications pour lesquelles les taxes ont été payées, à savoir les revendications n°s

4. ☐ Aucune taxe additionnelle demandée n'a été payée dans les délais par le déposant. En conséquence, le présent rapport de recherche internationale ne porte que sur l'invention mentionnée en premier lieu dans les revendications; elle est couverte par les revendications n°s

Remarque quant à la réserve

☒ Les taxes additionnelles étaient accompagnées d'une réserve de la part du déposant.

☐ Le paiement des taxes additionnelles n'était assorti d'aucune réserve.

SUITE DES RENSEIGNEMENTS INDIQUES SUR PCT/ISA/ 210

1. revendications: 2-10 et 1,14-23.28-32 partiellement

   ADNs caractérisés en ce qu'il comprennent une ou plusieurs séquence(s) tell(s) que présente(s) chez Neisseria meningitidis mais absente(s) chez Neisseria gonnorheae , cellule hôte , ARN , acides nucléiques anti-sens, polypeptides ,anticorps , méthode de diagnostic, kit de diagnostic , et composition vaccinale corrsepondants .

2. revendications: 11-13 et 1,14-23,28-32 partiellement

   ADNs caractérisés en ce qu'il comprennent une ou plusieurs séquence(s) tell(s) que présente(s) chez Neisseria meningitidis mais absente(s) chez Neisseria lactamica , cellule hôte , ARN , acides nucléiques anti-sens, polypeptides ,anticorps , méthode de diagnostic, kit de diagnostic , et composition vaccinale corrsepondants .

3. revendications: 24-27

   procédé d'obtention de banques d?ADN Neisseria meningitidis spécifiques comprenant le mélange d'une population de Neisseria meningitidis , avec une population de Neisseiria gonnorheae, soit de Neisseria lactamica , banques de clones d'ADN et leurs applications correspondantes .

RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

Demande internationale No
PCT/FR 97/01295

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
WO 9405703 A	17-03-94	AU 5093693 A	29-03-94
		CA 2122630 A	17-03-94
		EP 0625165 A	23-11-94
EP 0452596 A	23-10-91	AT 128189 T	15-10-95
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		EP 0525095 A	03-02-93
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RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

Dem. Internationale No
PCT/FR 97/01295

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
EP 0337896 A		ES 2058566 T	01-11-94
		JP 2203800 A	13-08-90
WO 9015621 A	27-12-90	AU 5851390 A	08-01-91

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/00135

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/22 A61K39/095 C07K16/12 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DELAHAY RM ET AL: "Involvement of the gonococcal MtrE protein in the resistance of Neisseria gonorrhoeae to toxic hydrophobic agents" MICROBIOLOGY, vol. 143, no. 7, July 1997 (1997-07), pages 2127-2133, XP000907381 figure 1 ---	1-16
X	FUSSENEGGER M ET AL.: "A novel peptidoglycan-linked lipoprotein (ComL) that functions in natural transformation competence of Neisseria gonorrhoeae" MOLECULAR MICROBIOLOGY, vol. 19, no. 5, 1996, pages 1095-1105, XP000907394 figure 2 --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2000	28 08 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	CUPIDO, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 02547 A (INST NAT SANTE RECH MED ;MAX PLANCK GESELLSCHAFT; SMITHKLINE BEECHAM) 22 January 1998 (1998-01-22) the whole document	1-18
A	MARTIN D ET AL: "HIGHLY CONSERVED NEISSERIA MENINGITIDIS SURFACE PROTEIN CONFERS PROTECTION AGAINST EXPERIMENTAL INFECTION" JOURNAL OF EXPERIMENTAL MEDICINE,JP,TOKYO, vol. 185, no. 7, 1997, pages 1173-1183-1183, XP000884332 ISSN: 0022-1007	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/00135

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9802547 A	22-01-1998	FR 2751000 A	16-01-1998
		AU 3697797 A	09-02-1998
		EP 0951552 A	27-10-1999

Applicant's or agent's file reference	International application No.
KP/BM45348	

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 110, line 2-22

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

10801 UNIVERSITY BLVD, MANASSAS, VIRGINIA 20110-2209,
UNITED STATES OF AMERICA

Date of deposit	Accession Number
22 June 1997 (22.06.97)	13090

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of those designations where a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European Patent or until the date on which the application has been refused or withdrawn, only by issue of such a sample to an expert nominated by the person requesting the sample.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
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